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09/355705

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
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I hereby certify that this paper of fee and the papers indicated as being attached hereto are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 C.F.R. §1.10 on the date indicated above and are addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Patricia Muñoz
(Typed or printed name of person mailing)

Patricia Muñoz
(Signature of person mailing)

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371		ATTORNEY DOCKET NUMBER 24743-2303US
INTERNATIONAL APPLICATION NO. PCT/US98/02007	INTERNATIONAL FILING DATE 04 February 1998	PRIORITY DATE CLAIMED 04 February 1997
TITLE: A REVERSIBLE STOICHIOMETRIC PROCESS FOR CONJUGATING BIOMOLECULES		
APPLICANTS FOR DO/EO/US: KÖSTER, Hubert, RUPPERT, Andreas		

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. [ ] This is a **SECOND** OR **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. §371(c)(2)):
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [X] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. §371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3)):
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11 to 16 concern other documents or information included:

11. [ ] An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.
12. [X] An unexecuted copy of a DECLARATION and POWER OF ATTORNEY with claim under 35 U.S.C. §119(e) for benefit of priority applications U.S. Application Serial No. 60/037,165.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items of information.
    Small Entity Statement

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APPLICATION NO. 09/355705	INTERNATIONAL APPLN. NO. PCT/US98/02007	ATTORNEY DOCKET NUMBER 24743-2303US

17. [X] The following fees are submitted:		CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO	$ 840.00		
International preliminary examination fee paid to PTO (37 CFR 1.482)	$ 670.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	$ 760.00		
Neither international preliminary examination fee paid to USPTO (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$970.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Articles 33(2)-(4)	$ 96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [X] 30 months for the earliest claimed priority date (37 CFR 1.492(e)).		$ 130.00	

Claims*	Number Filed	Number Extra	Rate		
Total claims	55	35	18	$ 630.00	
Independent claims	11	8	78	$ 624.00	
Multiple dependent claims		0	260	$ 0.00	

	CALCULATIONS	PTO USE ONLY
***BASED UPON ENTRY OF THE ATTACHED PRELIMINARY AMENDMENT** **TOTAL OF ABOVE CALCULATIONS =**	$ 2224.00	
Reduction by 1/2 for filing small entity	$ 1112.00	
SUBTOTAL =	$ 1112.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months for the earliest claimed priority date (37 CFR 1.492(f)).		
TOTAL NATIONAL FEE =	$ 1112.00	
TOTAL FEES ENCLOSED =	$ 1112.00	
	Amount to be: refunded	$
	charged	$

a. [X] A check in the amount of $1112.00 to cover the above fees is enclosed.

b. [X] Please charge Deposit Account No. 08-1641 for the above fees or for any amount due that is not covered by the enclosed check or if the enclosed check is in the wrong amount, post-dated or otherwise improper. A duplicate of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any other fees that may be required, or credit any overpayment to Deposit Account No. 08-1641.

SEND ALL CORRESPONDENCE TO:

Stephanie Seidman
Heller Ehrman White & McAuliffe
4250 Executive Square, 7th Floor
La Jolla, CA 92037
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Stephanie Seidman

514 Rec'd PCT/PTO 09/355705
0 3 AUG 1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: KÖSTER et al.

National Stage of International Appln. No.:
PCT/US98/02007
Filed: February 4, 1998

Filed: herewith (August 3, 1999)

For: A REVERSIBLE STOICHIOMETRIC PROCESS FOR CONJUGATING BIOMOLECULES

Group Art Unit: Unassigned

Examiner: Unassigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to the examination of the above-captioned application, please amend the application as follows:

IN THE SPECIFICATION:

at page 1, before "Background of the Invention", please insert the following:

—Related Applications

This application is related U.S. provisional application Serial No. 60/037,165, entitled "A Reversible Stoichiometric Process for Conjugating Biomolecules", filed February 4, 1997. Benefit of priority under 35 U.S.C. §119(e) is claimed to thereto and the subject matter of the provisional application is incorporated by reference in its entirety.— and

at page 5, line 24, replace "Sephadex, Sepharose" with —dextran cross-linked with epichlorohydrin (Sephadex^R), agarose (Sepharose^R)—.

IN THE CLAIMS:

Please cancel claim 52 without prejudice or disclaimer.

Please add claims 53-56 as follows:

—53. The insoluble support of claim 16, wherein the polypeptide is an enzyme.—

—54. The insoluble support of claim 53, wherein the enzyme is an alkaline phosphatase.—

—55. The insoluble support of claim 54, wherein the enzyme is bacterial alkaline phosphatase (BAP).—

—56. The insoluble support of claim 1, wherein the reversible linkages are different.—

Please amend claims 1-36, 40, 41 and 44-51 as follows:

1. (Amended) [A composition comprised of at least] An insoluble support, comprising two biopolymers, [conjugated to an insoluble support by at least one reversible linkage] wherein:

the first biopolymer is linked to the support by a reversible linkage; and

the second biopolymer is linked to the first biopolymer by a reversible linkage.

2. (Amended) [A composition according to] The insoluble support of claim 1, wherein the [at least] two biopolymers are comprised of nucleic acids.

3. (Amended) [A composition according to] The insoluble support of claim 1, wherein the [at least] two biopolymers are comprised of polypeptides.

4. (Amended) [A composition according to] The insoluble support of claim 1, wherein the [at least] two biopolymers are comprised of a nucleic acid and a protein.

5. (Amended) [A composition according to] The insoluble support of claim 1, wherein [the at least] one reversible linkage is formed through a trityl derivative, a chelate complex, a hydrophobic interaction or a photocleavable functionality.

6. (Amended) [A composition according to] The insoluble support of claim 1, wherein the insoluble support is selected from the group consisting of[:] a flat surface, a microtiter plate, a comb and a bead.

7. (Amended) [A composition according to] The insoluble support of claim 6, wherein the insoluble support is selected from the group consisting of[:] a silicon wafer, glass plate, metal, plastic, film and composites thereof with pits or wells.

8. (Amended) [A composition according to] The insoluble support of claim 7, further comprising two or more additional linked biopolymers, wherein the [biopolymer is conjugated] biopolymers are linked to the insoluble support in an array format.

9. (Amended) [A composition according to] The insoluble support of claim 7, wherein the [bead is comprised of] support comprises an inorganic material selected from the group consisting of[:] silica, Controlled Pore Glass (CPG), plastic, metal, cellulose, [Sepharose and Sephadex] agarose and dextran cross–linked with epichlorohydrin.

10. (Amended) [A composition according to] The insoluble support of claim 6, wherein the insoluble support [is comprised of] comprises a magnetic or electromagnetic material.

11. (Amended) [A composition according to] The insoluble support of claim 2, wherein the nucleic [acid is] acids are selected from the group consisting of[:] deoxyribonucleic acid (DNA), ribonucleic acid (RNA) [or] and analogs or mimetics of DNA or RNA.

12. (Amended) [A composition according to] The insoluble support of claim 3, wherein the [polypeptide is] polypeptides are selected from the group consisting of an antibody, enzyme, receptor [or] and peptide.

13. (Amended) [A composition according to] The insoluble support of claim 1, [which contains] further comprising a spacer between the first biopolymer and the insoluble support.

14. (Amended) [A composition according to] The insoluble support of claim 4, [which is made by the formation of a chelate complex between the nucleic acid and the polypeptide] wherein the reversible linkage between the nucleic acid and the polypeptide comprises a chelate complex.

15. (Amended) [A composition according to] The insoluble support of claim 14, wherein the chelate complex is formed by the reaction of a nucleic acid containing a chelate functionality with a polypeptide containing an [imidazoyl] imidazolyl functionality in the presence of a metal ion.

16. (Amended) [A composition] The insoluble support of claim 14, wherein the chelate complex is formed by the reaction of a nucleic acid containing an [imidazoyl] imidazolyl functionality with a polypeptide containing a chelate functionality in the presence of a metal ion.

17. (Amended) [A composition according to] The insoluble support of claim 15 [or 16], wherein the polypeptide is an enzyme.

18. (Amended) [A composition according to] The insoluble support of claim 17, wherein the enzyme is an alkaline phosphatase.

19. (Amended) [A method according to] The insoluble support of claim 18, wherein the enzyme is bacterial alkaline phosphatase (BAP).

20. (Amended) A method for [making a composition] preparing the insoluble support of claim 1, comprising the steps of:

a) immobilizing a nucleic acid to an insoluble support via a first reversible linkage; and

b) conjugating said nucleic acid with a polypeptide via a second reversible linkage.

21. (Amended) [A method according to] The method of claim 20, wherein the first or second reversible linkage is formed through a trityl derivative, a chelate complex, a hydrophobic interaction or a photocleavable functionality.

22. (Amended) A method according to claim 20, wherein in step b), the [first or] second reversible linkage forms a chelate complex.

23. (Amended) [A method according to] The method of claim 22, wherein the first or second reversible linkage is formed by the reaction of a nucleic acid containing a chelate functionality with a polypeptide containing an [imidazoyl] imidazolyl functionality in the presence of a metal ion.

24. (Amended) [A method according to] The method of claim 22, wherein the first or second reversible linkage is formed by the reaction of a nucleic acid containing an [imidazoyl] imidazolyl functionality with a polypeptide containing a chelate functionality in the presence of a metal ion.

25. (Amended) [A method according to] The method of claim 20, wherein the first or second reversible linkage [are] is formed from functionalities or precursors, which are introduced into the nucleic acid during enzymatic synthesis.

26. (Amended) [A method according to] The method of claim 25, wherein the enzymatic synthesis is part of an amplification procedure.

27. (Amended) [A] The method of claim 26, wherein the amplification procedure is selected from the group consisting of the polymerase chain reaction (PCR), the ligase chain reaction (LCR) and strand displacement amplification (SDA).[.]

28. (Amended) [A method according to] The method of claim 25, wherein the enzymatic synthesis is part of a nucleic acid sequencing procedure.

29. (Amended) An oligonucleotide analog [comprised of] comprising a β-cyanoethylphosphoamidite functionality [with] linked to a chelate functionality.

30. (Amended) [An] The oligonucleotide analog of claim 29, wherein the chelate functionality is a precursor of nitrilotriacetic acid derived from [either] serine, cysteine or lysine.

31. (Amended) An oligonucleotide analog, [comprised of] comprising a heterobifunctional trityl group [with] linked to a chelate functionality.

32. (Amended) [An] The oligonucleotide analog of claim 31, wherein the chelate functionality is a precursor of [nitrilotrisacetic] nitrilotriacetic acid derived from serine, cysteine or lysine.

33. (Amended) An oligonucleotide analog, [comprised of] comprising a β-cyanoethylphosphoamidite functionality [with] linked to an imidazolyl functionality.

34. (Amended) An oligonucleotide analog, [comprised of] comprising a heterobifunctional trityl group [with] linked to [a] an oligohistidyl or oligoimidazolyl sequence.

35. (Amended) [An] The oligonucleotide analog [according to] of claim 34, wherein the oligohistidyl sequence is present at the 5'- or 3'- terminus.

36. (Amended) An oligonucleotide analog, [comprised of] comprising an imidazolylnucleoside-β-cyanoethylphosphoamidite.

40. (Amended) [A] The recombinant protein [according to] of claim 39 which has enzymatic activity.

41. (Amended) [A] The recombinant protein [according to] of claim 40, which is an alkaline phosphatase that comprises[, which has] an alanine residue at its N-terminus instead of arginine-threonine and [which has] at its C-terminus a chain of [six] histidine residues.

44. (Amended) [A composition] The insoluble support of claim 1, wherein:

the first biopolymer is a nucleic acid;

the insoluble support is linked via a spacer to the nucleic acid through a reversible heterobifunctional trityl group;

the second biopolymer is an enzyme; and

the nucleic acid is conjugated to [an] the enzyme through a reversible chelate [functionality] complex.

45. (Amended) [A composition according to] The insoluble support of claim 44 in which the [polymer] insoluble support is comprised of magnetic beads[,] ; the chelate complex is formed via [the] a nitrilotriacetic acid functionality in the presence of Ni^{2+} ; and the enzyme is BAP-his_6.

46. (Amended) [A composition according to] The insoluble support claim 44 in which the [polymer] insoluble support is a silicon wafer carrying the reversible functionalities to bind the nucleic acid either directly on the surface or through beads in pits or wells in an array format[,] ; the chelate complex is formed via [nitrilotrisacetic] a nitrilotriacetic acid functionality in the presence of Ni^{2+} ; and the enzyme is BAP-his_6.

47. (Amended) [A composition according to] The insoluble support of claim 44 in which the [polymer] insoluble support is the filter bottom in the wells of a microtiter filter plate[,]; the chelate complex is formed via [nitrilotrisacetic] a nitrilotriacetic acid functionality in the presence of Ni^{2+} ; and the enzyme [if] is BAP-his_6.

48. (Amended) A method of purification, comprising:
[of using the composition according to] contacting the insoluble support of claim 44[to] with [purify and to detect] products of nucleic acid amplification procedures, whereby the products are purified.

49. (Amended) [A] The method of claim 48, wherein the amplification procedure is selected from the group consisting of[:] the polymerase chain reaction, the ligase chain reaction and strand displacement amplification.

50. (Amended) A method of sequencing a target nucleic acid, [for using the composition according to] comprising sequencing target nucleic acid wherein nucleic acid bound to the insoluble support of claim 44 serves as a primer[for determining the sequence of a nucleic acid].

51. (Amended) A method for genetic or expression profiling, comprising [for using the composition according to] contacting the insoluble support of claim 44 with a sample comprising mRNA or cDNA, thereby detecting [to purify and to detect] the identity and relative quantity of the mRNA or cDNA [mRNAs or their corresponding cDNAs for genetic or expression profiling].

REMARKS

Claims 1-51 and 53-56 are presently pending. Claims 1-36, 40, 41 and 44-51 are amended herein. The claims are amended herein to delete multiple dependencies, to ensure proper dependency, and to correct obvious typographical and other obvious errors. Basis for the amendment to claim 1 herein may be found in the specification, for example, at page 5, line 11 through page 7, line 24, and in Figures 1-3.

Claim 52 is cancelled herein without prejudice or disclaimer. Applicant reserves the right to file divisional applications to any cancelled subject matter.

Claim 9 is amended to remove trademarks, and to replace them with their generic equivalents.

Similarly, the specification is amended to identify trademark items as such (e.g., Sephadex and Sepharose represented as Sephadex^{R} and Sepharose^{R}, respectively), and to provide generic equivalents for trademark items (Sephadex^{R} and Sepharose^{R}) on page 5, line 24. These items are well-known to individuals skilled in the art and their generic descriptions are readily available. Pages from

a trade catalog describing the trademarked items and their generic equivalents are provided. Therefore, no new matter has been added.

Claims 53-56 are added herein. Basis for these claims may be found, for example, in claim 17 as originally filed. Therefore, no new matter has been added.

* * *

Entry of the above amendments and examination of the application are respectfully requested.

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE

By:
Stephanie Seidman
Registration No. 33, 779

Attorney Docket No. 24743-2303US

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: KÖSTER <u>et al.</u>

National Stage of International Appln. No.: PCT/US98/02007
Filed: February 4, 1998

Filed: herewith (August 3, 1999)

For: A REVERSIBLE STOICHIOMETRIC PROCESS FOR CONJUGATING BIOMOLECULES

Group Art Unit: Unassigned

Examiner: Unassigned

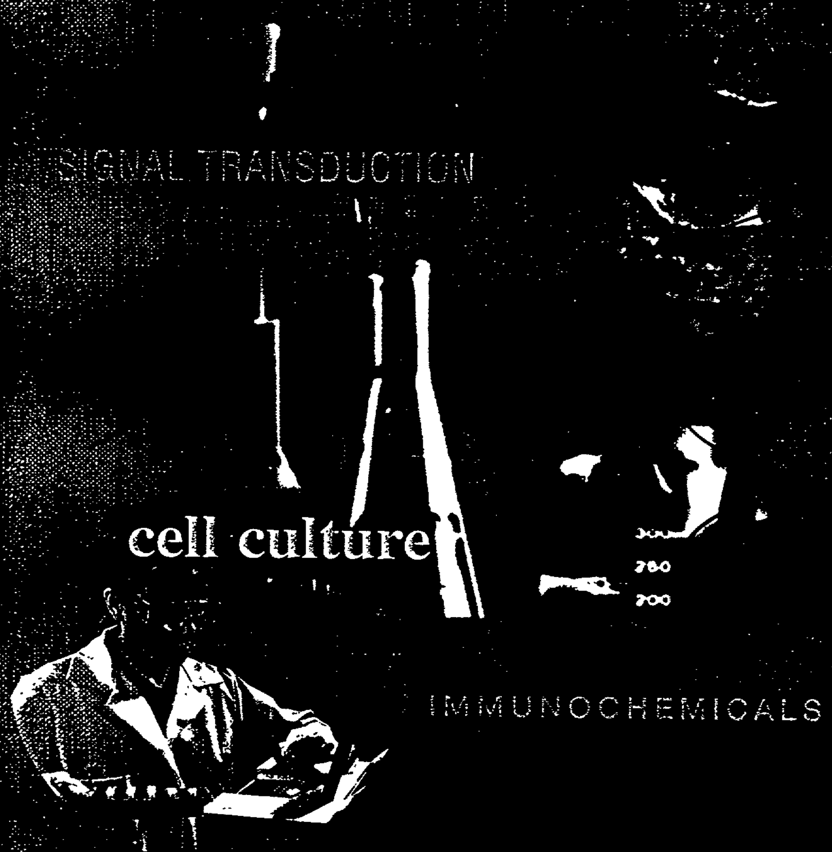
ATTACHMENTS TO THE PRELIMINARY AMENDMENT

1. Sigma Catalog (1998); pages 1901 and 1903, containing listings for Sephadex^R and Sepharose^R, respectively.

BIOCHEMICALS AND REAGENTS FOR LIFE SCIENCE RESEARCH

1998

Molecular Biology



SIGMA®

NEW PRODUCTS

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DIAGNOSTIC KITS AND REAGENTS

PRODUCT INDEX

[FIL]TRATION

f sample molecules to enter pores of a stationary phase. Very gh the chromatographic bed fastest. Above a certain size (the n occurs. Very small molecules easily enter the stationary phase cules move equally slowly, and again, no separation occurs. Bet- rying extents, depending on their size and shape. Within this ran- rates, and separation occurs.

also commonly used in rapid desalting procedures and in s have the smallest pores, with exclusion limits ranging from s of media range from 100,000 to 100,000,000. Applications iruses. Within groups, the choice is made by particle size. ies, but poorer resolution. Before selecting or using any product information.

/ (× 10³)	Bead Diameter (μm)		Approx. bed volume (ml/g)
xtrans	Dry	Wet	
drin under alkaline conditions			
:0.7	40-120	55-166	2-3
:1.5	40-120	60-181	2.5-3.5
).1-5	100-300	172-516	4-6
).1-5	50-150	86-258	4-6
).1-5	20-80	34-138	4-6
).1-5	20-50	17-69	4-6
.5-10	100-300	202-606	9-11
.5-10	50-150	101-303	9-11
.5-10	20-80	40-160	9-11
.5-10	20-50	20-80	9-11
1-50	40-120	92-277	12-15
1-50	20-50	23-92	12-15
i-100	40-120	103-311	15-20
i-100	20-50	26-103	15-20
;-150	40-120	116-349	20-30
1-150	20-50	29-116	18-22
1-200	40-120	129-388	30-40
1-200	20-50	32-129	20-25
—	50-150	—	4-6
—	100-300	—	4-6
—	50-150	—	9-11
—	100-300	—	9-11
is(acrylamide)			
—	—	25-75	Preswollen
1-80	—	25-75	Preswollen
1-400	—	25-75	Preswollen
0-2000	—	25-75	Preswollen
)-20,000	—	25-75	Preswollen
)-100,000	—	40-105	Preswollen
ules of high molecular weight			
0-1000	—	45-165	Preswollen
i0-1000	—	40-165	Preswollen
)0-5000	—	45-165	Preswollen
)0-5000	—	40-165	Preswollen
)0-20,000	—	60-200	Preswollen
0-20,000	—	60-200	Preswollen
0.5-30	—	24-44	Preswollen
1-100	—	24-44	Preswollen
—	—	20-40	Preswollen
—	—	20-40	Preswollen
lamide) and a hydroxylated cross-linker; resistant to acid but sensitive to alkali;			
—	—	40-80	Preswollen
—	—	80-160	Preswollen
de)			
—	—	40-80	Preswollen

GEL FILTRATION

Product Number	Name	Fractionation Range, MW (× 10³)		Bead Diameter (μm)		Approx bed volume (ml/g)
		Peptides, Globular proteins	Dextrans	Dry	Wet	
Ultrogel A — Beaded agarose, free of fines						
U 0382	A2	120-25,000	—	—	60-140	Preswollen
U 0507	A4	55-9,000	—	—	60-140	Preswollen
U 0632	A6	25-2,400	—	—	60-140	Preswollen
Ultrogel AcA — Beaded composite polyacrylamide/agarose, stable between pH 3 and 10						
U 0257	AcA 22	100-1,200	—	—	60-140	Preswollen
U 8878	AcA 34	20-350	—	—	60-140	Preswollen
U 8753	AcA 44	10-130	—	—	60-140	Preswollen
U 8628	AcA 54	5-70	—	—	60-140	Preswollen
Beaded Cellulose — highly porous regenerated cellulose beads						
C 7079	90% porosity	10-1000	—	—	30-50	Preswollen
C 7204	90% porosity	10-1000	—	—	50-80	Preswollen
C 7329	90% porosity	10-1000	—	—	80-100	Preswollen
C 7454	90% porosity	10-1000	—	—	100-250	Preswollen
C 7579	90% porosity	10-1000	—	—	250-500	Preswollen
C 7704	93% porosity	100-3000	—	—	50-80	Preswollen
C 7829	93% porosity	100-3000	—	—	80-100	Preswollen
C 7954	93% porosity	100-3000	—	—	100-250	Preswollen
C 8079	93% porosity	100-3000	—	—	250-500	Preswollen
C 8204	95% porosity	2000-5000	—	—	50-80	Preswollen
C 8329	95% porosity	2000-5000	—	—	80-100	Preswollen
C 8454	95% porosity	2000-5000	—	—	100-250	Preswollen
C 8579	95% porosity	2000-5000	—	—	250-500	Preswollen

PRODUCT NUMBER | US $ | PRODUCT NUMBER

SEPHADEX (SIGMA CATALOG)
Beads for gel filtration prepared by cross-linking dextran with epichlorohydrin
See also: Sephadex DNA Grade in the Molecular Biology Section *Page 1670*

G-10-120 RT **Sephadex G-10**
Fractionation range (MW)
Globular proteins: <700
Dextrans: <700
Dry bead diameter: 40-120 μm
Bed volume: 2-3 ml per g
[9050-68-4]
10 g 20.80; 50 g 69.30; 100 g 115.50; 500 g 525.00

G-15-120 RT **Sephadex G-15**
Fractionation range (MW)
Globular proteins: <1500
Dextrans: <1500
Dry bead diameter: 40-120 μm
Bed volume: 2.5-3.5 ml per g
[11081-40-6]
10 g 20.80; 50 g 69.30; 100 g 115.50; 500 g 525.00

G-25-300 RT ***Sephadex G-25 Coarse***
Fractionation range (MW)
Globular proteins: 1000-5000
Dextrans: 100-5000
Dry bead diameter: 100-300 μm
Bed volume: 4-6 ml per g
[9041-35-4]
10 g 20.80; 50 g 69.30; 100 g 115.50; 500 g 525.00

G-25-150 RT **Sephadex G-25 Medium**
Fractionation range (MW)
Globular proteins: 1000-5000
Dextrans: 100-5000
Dry bead diameter: 50-150 *μm*
Bed volume: 4-6 ml per g
[9041-35-4]
10 g 20.80; 50 g 61.80; 100 g 115.50; 500 g 525.00

(Continued)

(Continuation of)
SEPHADEX

5-4805 RT **PD-10 Column** 30 / pkg 131
Sephadex G-25 Medium packed in 9.1 ml (15 × 50 mm) polypropylene column
Max. pressure: 170 psi (1.2 MPa)
Max. sample volume: 2.5 ml
Typical desalting time: 5 min

G-25-80 RT ***Sephadex G-25 Fine***
Fractionation range (MW)
Globular proteins: 1000-5000
Dextrans: 100-5000
Dry bead diameter: 20-80 μm
Bed volume: 4-6 ml per g
[9041-35-4]
10 g 20.; 50 g 69.; 100 g 115.; 500 g 525.

G-25-50 RT **Sephadex G-25 Superfine**
Fractionation range (MW)
Globular proteins: 1000-5000
Dextrans: 100-5000
Dry bead diameter: 20-50 μm
Bed volume: 4-6 ml per g
[9041-35-4]
10 g 28.; 50 g 94.; 100 g 157.

5-4804 RT **Fast Desalting Column HR10/10** 525.
Sephadex G-25 Superfine packed in 7.8 ml (10 × 100 mm) glass column
Max. flow/pressure: 6 ml/min at 170 psi (1.2 MP
Max. sample volume: *1.5 ml*
Typical desalting time: 1-4 min

G-50-300 RT **Sephadex G-50 Coarse**
Fractionation range (MW)
Globular proteins: 1500-30,000
Dextrans: 500-10,000
Dry bead diameter: *100-300 μm*
Bed volume: 9-11 ml per g
[9048-71-9]
10 g 22; 50 g 75; 100 g 126

(Continue

PRODUCT NUMBER | US $

(Continuation of)
SEPHADEX

150-50 **Sephadex G-150 Superfine** 10 g 47.25; 50 g 157.50; 100 g 262.50
Fractionation range (MW)
Globular proteins: 5000-150,000
Dextrans: 1000-150,000
Dry bead diameter: 20-50 μm
Bed volume: 18-22 ml per g
[12774-36-6]

200-120 **Sephadex G-200** 10 g 39.70; 50 g 132.30; 100 g 220.50
Fractionation range (MW)
Globular proteins: 5000-600,000
Dextrans: 1000-200,000
Dry bead diameter: 40-120 μm
Bed volume: 30-40 ml per g
[9041-36-5]

200-50 **Sephadex G-200 Superfine** 10 g 51.05; 50 g 170.10; 100 g 283.50
Fractionation range (MW)
Globular proteins: 5000-250,000
Dextrans: 1000-200,000
Dry bead diameter: 20-50 μm
Bed volume: 20-25 ml per g
[9041-36-5]

See also: Sephadex DNA Grade in the Molecular Biology Section *Page 1670*

PDX
Cross-linked dextran beads for Gel Filtration Chromatography

2676 **G.F. 25 (50-150)** 10 g 18.15
Fractionation range (MW)
Globular proteins: 1000-5000
Dry bead diameter: 50-150 μm
Bed volume: 4-6 ml per g
[9041-35-4]

2801 **G.F. 25 (100-300)** 10 g 19.00; 50 g 63.25; 100 g 105.35; 500 g 489.10
Fractionation range (MW)
Globular proteins: 1000-5000
Dry bead diameter: 100-300 μm
Bed volume: 4-6 ml per g
[9041-35-4]

6426 **G.F. 50 (50-150)** 10 g 17.55
Fractionation range (MW)
Globular proteins: 1500-30,000
Dry bead diameter: 50-150 μm
Bed volume: 9-11 ml per g
[9048-71-9]

6551 **G.F. 50 (100-300)** 100 g 113.40
Fractionation range (MW)
Globular proteins: 1500-30,000
Dry bead diameter: 100-300 μm
Bed volume: 9-11 ml per g
[9048-71-9]

PRODUCT NUMBER | US $

SEPHACRYL
Cross-linked co-polymer of allyl dextran and N,N′-methylenebisacrylamide for gel filtration
Suspension in 20% ethanol
The HR grades are smaller particle sizes with narrower size distributions optimized for more efficient separations and faster flow.

S-100-HR [2-8°C] **Fractionation range (MW)** 100 ml 67.35; 250 ml 134.70; 750 ml 299.25
Globular proteins: 1000-100,000
Wet bead diameter: 25-75 μm
R: 10-36/37/38 S: 16-26-36

S-200-HR [2-8°C] **Fractionation range (MW)** 100 ml 67.35; 250 ml 134.70; 750 ml 299.25
Globular proteins: 5000-250,000
Dextrans: 1000-80,000
Wet bead diameter: 25-75 μm
R: 10-36/37/38 S: 16-26-36

S-300-HR [2-8°C] **Fractionation range (MW)** 100 ml 67.35; 250 ml 134.70; 750 ml 299.25
Globular proteins: 10,000-1,500,000
Dextrans: 1000-400,000
Wet bead diameter: 25-75 μm
R: 10-36/37/38 S: 16-26-36

S-400-HR [2-8°C] **Fractionation range (MW)** 100 ml 67.35; 250 ml 134.70; 750 ml 299.25
Globular proteins: 20,000-8,000,000
Dextrans: 10,000-2,000,000
Wet bead diameter: 25-75 μm
R: 10-36/37/38 S: 16-26-36

S-500-HR [2-8°C] **Fractionation range (MW)** 100 ml 67.35; 250 ml 134.70; 750 ml 299.25
Dextrans: 40,000-20,000,000
Wet bead diameter: 25-75 μm
R: 10-36/37/38 S: 16-26-36

S-1000 [2-8°C] **Fractionation range (MW)** 100 ml 71.95; 500 ml 239.85; 750 ml 330.50
Dextrans: 500,000-100,000,000
Wet bead diameter: 40-105 μm
[84593-66-8]
R: 10-36/37/38 S: 16-26-36

SEPHAROSE (SIGMA CATALOG)
Beaded agarose for fractionating molecules of high molecular weight. Cross-linked beaded agarose is more resistant to denaturing conditions, and thus offers more versatility in the choice of sample buffer and eluent. The approximate % agarose concentration is indicated by the first number of the Stock Number.

6B-100 [2-8°C] **Sepharose 6B** 100 ml 61.45; 500 ml 204.75; 1 liter 341.25
Fractionation range (MW)
Globular proteins: 10,000-4,000,000
Dextrans: 10,000-1,000,000
Wet bead diameter: 45-165 μm
[9012-36-6]
R: 10-36/37/38 S: 16-26-36

CL-6B-200 [2-8°C] **Sepharose CL-6B (cross-linked)** 100 ml 70.90; 500 ml 236.25; 1 liter 393.75
Fractionation range (MW)
Globular proteins: 10,000-4,000,000
Dextrans: 10,000-1,000,000
Wet bead diameter: 40-165 μm
[62610-50-8]
R: 10-36/37/38 S: 16-26-36

(Continued)

(Continuation of)
SEPHAROSE

4B-200 [2-8°C] **Sepharose 4B** 100 ml 58.60; 500 ml 195.30; 1 liter 325.50
Fractionation range (MW)
Globular proteins: 60,000-20,000,000
Dextrans: 30,000-5,000,000
Wet bead diameter: 45-165 μm
[9012-36-6]
R: 10-36/37/38 S: 16-26-36

CL-4B-200 [2-8°C] **Sepharose CL-4B (cross-linked)** 100 ml 65.25; 500 ml 217.35; 1 liter 362.25
Fractionation range (MW)
Globular proteins: 60,000-20,000,000
Dextrans: 30,000-5,000,000
Wet bead diameter: 40-165 μm
[61970-08-9]
R: 10-36/37/38 S: 16-26-36

2B-300 [2-8°C] **Sepharose 2B** 100 ml 58.60; 500 ml 195.30; 1 liter 325.50
Fractionation range (MW)
Globular proteins: 70,000-40,000,000
Dextrans: 100,000-20,000,000
Wet bead diameter: 60-200 μm
[9012-36-6]
R: 36/37/38 S: 26-36

CL-2B-300 [2-8°C] **Sepharose CL-2B (cross-linked)** 100 ml 62.40; 500 ml 207.90; 1 liter 346.50
Fractionation range (MW)
Globular proteins: 70,000-40,000,000
Dextrans: 100,000-20,000,000
Wet bead diameter: 60-200 μm
[65099-79-8]
R: 10-36/37/38 S: 16-26-36

[RT] **SigmaChrom GFC GEL FILTRATION HPLC COLUMNS** 655.00
For analysis and purification of peptides and proteins by high-performance GFC. SigmaChrom GFC columns are packed with a composite matrix of cross-linked polysaccharides, 12-15 μm bead diameter. The matrix is stable in the pH range 3-12. Columns are 7.5 mm I.D. × 300 mm long (13.3 ml bed volume); all parts that come in contact with the sample are constructed of PEEK, a biocompatible plastic. Columns can be operated up to 0.9 ml/min at 250 psi back pressure. Up to 5.5 mg of protein can be separated without overloading.

5-4750 **SigmaChrom™ GFC-100 Column**
Exclusion limit (globular proteins): 100,000

5-4751 **SigmaChrom™ GFC-1300 Column**
Exclusion limit (globular proteins): 1,300,000

SUPERDEX
Composite of cross-linked agarose and dextran
pH Range: 3-12 (long term); 1-14 (short term)

S 2792 [2-8°C] **Superdex 30 Prep Grade** 25 ml 77.35; 100 ml 214.25
Fractionation Range (MW)
Globular proteins: up to 10,000
Bead diameter: 24-44 μm.
Suspension in 20% ethanol
R: 10-36/37/38 S: 16-26-36

(Continued)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: KÖSTER et al.

US Appl. No.: 09/355,705
US Filing Date: August 3, 1999

Intl. Appl. No.: PCT/US98/02007
Intl. Filing Date: February 4, 1998

For: *A REVERSIBLE STOICHIOMETRIC PROCESS FOR CONJUGATING BIOMOLECULES*

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as first class mail in an envelope addressed to:
Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231, on this date.

11/19/1999
Date

Kathy Holloway

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to the examination of the above-captioned application, please amend the application as follows:

IN THE SPECIFICATION:

at page 2, line 5, after "established" and before "due" replace "," with -. This is-.

at page 5, line 15, after "polypeptides)" and before "to" remove "."

at page 5, line 17, replace "soluble supports" with -solid supports-.

at page 7, line 1, replace "fish" with -select-.

at page 7, line 11, replace "5,547,035" with -5,547,835-.

at page 9, line 5, replace "moelcule" with -molecule-.

National Stage of International Appln. No.: PCT/US98/02007
KÖSTER et al.
PRELIMINARY AMENDMENT

REMARKS

The specification is amended to remove grammatical and typographical errors. Therefore, no new matter has been added.

* * *

Entry of the above amendments and examination of the application are respectfully requested.

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE

By: ______________________
Stephanie Seidman
Registration No. 33, 779

Attorney Docket No. 24743-2303US
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Attorney Docket No. 24743-2303US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Hubert Köster and Andreas Ruppert

Serial No.: not assigned

Filed: Herewith

For: **A REVERSIBLE STOICHIOMETRIC PROCESS FOR CONJUGATING BIOMOLECULES**

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§1.9(f) and 1.27(b) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 C.F.R. §1.9(c) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled

A REVERSIBLE STOICHIOMETRIC PROCESS FOR CONJUGATING BIOMOLECULES

described in

(X) the specification filed herewith
( ) application Serial No. ______________, filed ______________
( ) Patent No. __________________, issued ____________________

I have not assigned, granted, conveyed, or licensed and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 C.F.R. §§1.9 (c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 C.F.R. §§1.9(d) or a nonprofit organization under 37 C.F.R. §1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 C.F.R. §1.27).

Full Name N/A ____
Address ____
( ) Individual ( ) Small Business Concern ( ) Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 C.F.R. §1.28(b)).

Attorney Docket No. 24743-2303US

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF INVENTOR: HUBERT KÖSTER

SIGNATURE OF INVENTOR: [signature]

DATE: 8/2/99

A Reversible Stoichiometric Process for Conjugating Biomolecules

Background of the Invention

Methods for reversibly linking biomolecules (e.g. nucleic acids with reporter groups or to solid supports) is important for many applications in the life sciences; it is used amongst other applications in DNA sequencing, DNA diagnostics, nucleic acid purification, Polymerase and Ligase Chain Reactions (PCR, LCR), hybridization experiments and solid phase biochemistry. Most frequently, a reversible linkage is accomplished via a streptavidin-biotin interaction (L.G. Mitchel and C.R. Merril (1989) *Anal. Biochem.*, 178, 239-242; B.H. Bowman and S.R. Palumbi (1993) in E.A. Zimmer, R.L. Cann and A.C. Wilson (ed.) *Methods of Enzymology*, Academic Press, New York, Vol. 224, pp. 399-405; X. Tong and L.M. Smith (1992) *Anal. Chem.* 64, 2672-2677).

Another reversible linkage, which is particularly amenable for linkage of nucleic acids, can be accomplished via heterobifunctional trityl groups, which can be cleaved under acidic conditions (E. Leikauf, F. Barnekow and H. Köster, Heterobifunctional Trityl Derivatives as Linking Agents for the Recovery of Nucleic Acids after Labeling and Immobilization (1995) *Tetrahedron* 51, 3793-3802; H. Köster, J.M. Coull and B. Gildea, Succinimidyl Trityl Compounds and a Process for Preparing Same, Protecting Groups for Natural Products, US Patent 5,410,068).

The interaction of metal chelates with polypeptide sequences such as oligohistidine has been used for affinity chromatography of proteins (J. Porath (1992) *Protein Express Purif.* 3, 263-281; M.C. Smith et al. (1988) *J. Biol. Chem.*, 263, 7211-7215; E. Hochuli and S. Piesecki (1992) *Methods*, 4, 68-72; E. Huchuli et al. (1988) *BioTechnology 6*, 1321-1325; E. Blum et al. (1994) *Biochem. Biophys. J.* 29, 113-121; see also European Patent No. 0 253 303 to Hoffman LaRoche AG), nucleic acids (Ch. Min and G. L. Verdine, Immobilized Metal Affinity Chromatography of DNA (1996) *Nucleic Acids Res.* 24, 3806-3810) and recently a system to detect proteins has been

introduced (Qiagen (1996): QIAexpress Detection System). Occasionally also disulfide bridges are used, which can be cleaved under reducing conditions.

However, in applications in which proteins (e.g. antibodies, enzymes) are to be linked to nucleic acids (i.e. for the detection of nucleic acids), no specific and reproducible linkage to the nucleic acids can be established, due to the fact that during chemical functionalization or activation of functional groups on the surface of the protein, no precise selection of amino acid side chains is possible and therefore neither the attachment site nor the stoichiometry can be controlled. Therefore, the results obtained can be different from batch to batch which negatively influences the generation of quantitative nucleic acid detection systems. In addition, there is no control over whether the amino acid side chain is incorporated into the active site. These factors all reduce the technical value of such procedures.

The application of solid phase techniques simplifies the preparation and purification of the reaction products, which is important for subsequent analytical and biochemical procedures. Since in some cases cleavage of one of the products from the support is needed. e.g. for further biochemical reactions in solution or signal detection, a combination of at least two different reversible linkages cleavable under mild and selective conditions is needed.

<u>Summary of the Invention</u>

In one aspect, the invention features compositions comprised of at least two biopolymers (e.g. nucleic acids or polypeptides), which are conjugated to an insoluble support by two different reversible linkages, which are cleavable under selective conditions.

In another aspect, the invention features novel methods and components for specifically conjugating biomolecules under completely controlled stoichiometry based on the specific and strong interaction between chelators in the presence of metal ions. In one embodiment, imidazolyl moieties are introduced via the introduction of

histidine residues (e.g. oligo-His) into a polypeptide (e.g. by recombinant DNA techniques). The oligo-His polypeptide can then interact in the presence of a metal with a nucleic acid carrying a chelator functionality at a position which is exposed and does not interfere with Watson-Crick base pairing of the nucleic acid. In another embodiment, which is particularly well-suited for the attachment of biomolecules other than polypeptides or for the reversible immobilization of nucleic acid molecules, the nucleic acid can carry a series of imidazolyl functionalities in a format which makes them available for chelation and which does not interfere with Watson-Crick base pairing; in which case, the other conjugating partner molecule can carry the chelator functionality.

By combining this reversible concept with other reversible or irreversible linkages, novel biochemical formats including diagnostic assays are possible in which favorable solid phase procedures are coupled with sensitive detection principles.

Brief Description of the Figures

Figure 1 (a) and (c) pictorially depict two general approaches of the invention in which a spacer molecule, A, linked to a polymer support, P, forms a reversible linkage, I, to a nucleic acid or protein/peptide molecule, B, which itself is linked by another reversible linkage, II, to either a nucleic acid, protein/peptide or small molecule (e.g. reporter molecule). Linkage I can be a heterobifunctional trityl group or a hydrophobic interaction stable under aqueous conditions or a photocleavable bond and II can be a bond, which is generated through a chelate complex. The two parts which form the linkage can be reversed (I', II') as shown in (b) and (d).

Figure 2 schematically depicts a nucleic acid molecule, B, which is linked through a spacer, A, via a reversible linkage, I, to a polymer support, P. B interacts via Watson-Crick complementarity with a nucleic acid molecule, C, which in turn through another reversible linkage II allows interaction with a reporter functionality D which can be a protein (enzyme), a nucleic acid or a small detector molecule.

Figure 3 schematically depicts the same approach as in Figure 2 with the

exception that B is linked to the polymer support through a spacer A with a non-reversible linkage.

Figure 4: shows an example of the chelate complex formed between a six residue histidine (his_6) tail and nitrilotriacetic acid (NTA) in the presence of Ni^{2+}.

Figure 5 schematically depicts a reaction, wherein a synthesized, protected N,N-dicarboxymethyl-serine phosphoamidite is synthesized as a chemical building block to introduce the NTA functionality into synthetic oligonucleotides.

Figure 6 shows the synthesis of a chelate-linked oligonucleotide to a his_6-BAP (bacterially generated alkaline phosphatase) conjugate by use of the phosphoamidite chelate precursor.

Figure 7 shows the synthesis of a chelate-linked oligonucleotide to his_6-BAP conjugate via retritylation and subsequent substitution with a chelate building block.

Figure 8 shows the structure of imidazolyl phosphoamidite building blocks for the single or multiple addition of an imidazolyl moiety during chemical oligonucleotide synthesis.

Figure 9 depicts the introduction of an imidazolyl moiety through an imidazolylnucleoside phosphoamidite.

Figure 10 shows the introduction of multiple imidazolyl moieties through chemical peptide synthesis of oligohistidine onto an oligonucleotide during solid phase chemical synthesis of oligonucleotides.

Figure 11 shows the chelate modified uracil and adenine nucleoside triphosphates for the enzymatic introduction of chelate functionalities into nucleic acids. Corresponding derivatives can be envisioned for cytidine, guanine or modified

nucleosides.

Figure 12 shows imidazolyl modified uracil and adenine nucleoside triphosphates for the enzymatic introduction of imidazolyl moieties into nucleic acids. Corresponding derivatives can be envisioned for cytidine, guanine and modified nucleosides.

Figure 13 schematically depicts solid phase separation/detection using NHS-DMT oligonucleotides linked to a solid phase and subsequently linked to a BAP-his_6 detector molecule via the LCR (Ligase Chain Reaction).

Figure 14 schematically depicts the detection of Polymerase Chain Reaction (PCR) products via the process of the invention.

Detailed Description of the Invention

As shown in Figure 1, two different reversible linkages I and II (a,c), which could be positioned with their functionalities reversed (I',II'; b, d) are used to link "biomolecules" or "biopolymers" (i.e.organic molecules, including nucleic acids, peptides, polypeptides). to an insoluble support. The circled P represents an insoluble or solid support.

"Insoluble supports" or "soluble supports" as used herein can be flat such as membranes, glass plates, metals, plastic films and composites thereof with a homogeneously functionalized surface or functionalized to result in an array format including flat supports with pits, wells, combs, microtiter plates, microtiter filter plates; flat supports can also be magnetic or with an array shaped (checkered) magnetic field; solid supports can also be used as beads from different plastic materials, inorganic supports such as silica, GPG (Controlled Pore Glass), metal, different polymeric material, cellulose, Sephadex, Sepharose; the beads can be porous or non-porous, of different diameter and magnetic or non-magnetic. Also a combination of beads in the pits/wells of flat supports thus forming an array format can be employed.

Compound A can be a spacer, a nucleic acid sequence (or nucleic acid analog/mimetic) or a protein or peptide sequence, B can be a nucleic acid (or a nucleic acid analog/mimetic) or a peptide or protein, whereas C can be nucleic acid (or a nucleic acid analog/mimetic), protein/peptide or a small reporter molecule. As an example A is a spacer and I is a heterobifunctional trityl group which is coupled to a nucleic acid B; B carries a chelate functionality which interacts with the poly-his tail of a recombinant alkaline phosphatase (his_6-AP), which carries e.g. a sequence of six histidine residues at the C-terminal end of the polypeptide chain. If a chromogenic or fluorogenic substrate is added, for example, dephosphorylation generates color or light thereby providing a nucleic acid detection system. The advantage of this system is that the detection can be done either on the insoluble support or after releasing B from the support by cleavage of bond I (or I'). It is therefore possible to remove all side-products from a reaction by filtration due to the attachment to a solid phase before performing the analytical step in solution. This leads to a robust, reproducible performance.

Figure 2 shows schematically how amplification (e.g. polymerase chain reaction (PCR) or ligase chain reaction (LCR) products B-C can be captured specifically, purified and subsequently detected on the support or in solution. The first reversible linkage I (or I') e.g. a heterobifunctional trityl group anchors one strand of the LCR or PCR product via a spacer A to the support through an acid labile tritylether bond the precursor of which has been introduced by an appropriately functionalized primer during the LCR or PCR reaction. The strand C carries e.g. the chelate functionality also introduced by using an appropriately functionalized primer during PCR or LCR. The chelated moiety can then interact with a reporter functionality e.g. his_6-AP for subsequent detection and quantification of amplification product. B can also be a cDNA molecule which can be linked through its 5'-end to the polymer support. With appropriate primers, solid phase DNA sequencing can be performed. Considering an array format, this could be used for high throughput genetic and expression profiling experiments.

As shown in Figure 2, B could also be a specific (or oligo-dT) capture

sequence to fish mRNA. The cDNA can be directly synthesized since the capture sequence simultaneously can act as a primer for the RNA dependent DNA polymerase. The RNA can be removed, the cDNA purified by washing and filtration steps and either released or directly used for subsequent DNA sequencing. It can also be envisioned that the capture sequence while serving as a primer for the RNA dependent DNA polymerase can be used directly to generate sequencing ladders employing ddNTP's as terminators. After purification of the sequencing ladders by washing and filtration, the bond to the polymer support is cleaved and the purified sequencing ladders subjected to either gel electrophoretic or mass spectrometric separation (H. Köster et al., A Strategy for Rapid and Efficient DNA Sequencing by Mass Spectrometry, *Nature Biotech*, (1996) 14, 1123-1128; U.S. Patent No. 5, 547,035 to H. Köster; International Patent Application No. W094/21822 to H. Köster; and International Patent Application No. W096/29431 to H. Köster)

Figure 3 shows a simplified version of Figure 2 in that nucleic acid fragment B is immobilized through a non-reversible bond via a spacer A to the solid support whereas nucleic acid C carries the reporter functionality via a reversible linkage so that detection can be performed either on the support or in solution.

In Figures 1-3, biopolymer C or D could be synthetic peptides linked to an immobilized nucleic acid B or B-C respectively via a reversible linkage as described (heterobifunctional trityl, photocleavable, chelate, hydrophobic interaction) which is then detected by mass spectrometry. Various defined peptide sequences can form a specific mass tag which can be used as a specific nucleic acid identifier. Conversely specific nucleic acid sequences can be used as mass tags (specific identifiers) for proteins immobilized through a spacer A.

For use in the instant process, nucleic acids can be single stranded or double stranded polynucleotides (including oligonucleotides), whether natural or synthetic, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) or DNA/RNA hybrids, DNA containing ribonucleotides and/or dideoxyribonucleotides and

RNA containing deoxyribonucleotides. Also encompassed by the term "nucleic acid" are modified nucleotides (e.g. phosphorothioate modified) as well as nucleic acid mimetics or analogs, such as peptide nucleic acids (PNAs).

As used herein, the terms "protein", "polypeptide" or "peptide" are all used interchangeably to refer to gene products. Proteins can be antibodies, enzymes, receptor molecules; peptides could be of natural or synthetic origin with oligo-his tail, a functionality for hydrophobic interaction, a photocleavable functionality or chelator functionality and displaying different properties such as being adhesive or representing specific ligand-receptor or specific protease cleavage sites.

As used herein, the term oligo his tail or poly his tail refers to a chain of conjugated histidine residues. Preferred oligo his tails contain 2-10 histidine residues. Particularly preferred oligo his tails are in the range of about 4 to about 8 his residues. Reversible linkages can be formed by hydrophobic interaction between e.g. a trityl group (i.e. with long aliphatic alkyl chains) and a long aliphatic chain e.g. attached to a polymer support or a hydrophobic polymer surface such as that of polystyrene. Since most biochemical and molecular biological reactions are performed in aqueous solution such hydrophobic interaction might be of sufficient stability. Addition of organic solvents such as alcohols, acetonitrile, N.N-dimethylformamide and the like will destabilize (if necessary in conjunction with heat) the hydrophobic interaction and release the attached molecules.

A reversible linkage which can independently be addressed could also be a functionality which is cleavable under photolytic conditions (see e.g. J. Olejnik, E. Krzymanska-Olejnik and K.J. Rothschil, Photocleavable Biotin Phosphoramidites for 5'-End-labeling, Affinity purification and Phosphorylation of Synthetic Oligonucleotides (1996) *Nucleic Acids Res.*, 24, 361-366). If the wavelength needed for photocleavage is in the range of the laser wavelength used in MALDI mass spectrometry, this bond can be cleaved during mass spectrometric signal acquisition.

A reversible linkage can also be formed from a chelator functionality which interacts with another chelator (e.g oligo-imidazolyl or other oligopeptide moieties) in the presence of a metal ion. The term "chelator" refers to a single molecule, which comprises at least two Lewis basic atoms that are capable of associating simultaneously with a Lewis acidic atom, moelcule or ion-- either simple or complex. "Lewis base" is an art recognized term that refers to chemical moieties, which are capable of donating to another atom or moiety at least one pair of unshared electrons. Examples include uncharged functional groups such as alcohols, ethers, carbonyls, thiols, sulfides, amines, imines, and pyridine and imidazole nitrogens; and charged functional groups, such as alkoxides, thiolates, carboxylates and a variety of other anions. "Lewis acid" is an art recognized term that refers to chemical moieties, which are capable of accepting from another atom or moiety (e.g. a Lewis basic atom or moiety) at least one pair of unshared electrons. Examples of Lewis acid moieties include transition metal halides, with at least one vacant d orbital, alkali metal cations, alkaline-earth metal cations, and trivalent boron or aluminum compounds. A "bidentate chelator", "tridentate chelator" and "tetradentate chelator" refers to chelators comprising two, three and four Lewis basic moieties, respectively, capable of simultaneous donation of at least an equal number of unshared electron pairs to another atom, ion or moiety.

Figure 4 depicts a specific example in which the chelator functionality is a nitrilotriacetic acid (NTA) which coordinates with divalent metal cations such as Ni^{2+} and forms a strong complex with six imidazolyl groups from a his_6 tail linked to one of the conjugating partner molecules. The term "imidazolyl residue" or "imidazoyl group" refers to any substituted or unsubstituted form of imidazole (i.e. 1,3-diaza-2,4-cyclopentadiene). For example, the side chain of the amino acid histidine comprises an imidazolyl residue.

The determination of which of the two necessary functions is attached to the nucleic acid molecule or the protein depends on the ease and convenience of introduction of either functionality (e.g. NTA or his_6 tail). In case of proteins the site-specific introduction of a chelator molecule seems to be difficult whereas the his_6 tail can

be introduced through recombinant DNA technologies. In contrast to currently available procedures, for linking nucleic acids to proteins (e.g. chemical linkage using either maleimide-thiol coupling (S.S. Gosh et al. (1990), *Bioconjugate Chem.* 1, 71-76), disulfide bonds (B.C.F. Chu L.E. Orgel (1988) *Nucleic Acids Res.* 16, 3671-3691) or mediated via streptavidin, which binds both biotinylated nucleic acids and biotynylated alkaline phosphatase (AP) (J.J. Leary et al. (1983) *Proc. Natl. Acad. Sci.* USA 80, 4045-4049)), the introduction of the his_6 tail through recombinant DNA technologies allows site-specific introduction.

As an example which does not limit the scope of this invention, the process is explained for alkaline phosphatase (AP) as protein. Alkaline phosphatase (EC 3.1.3.1) is a versatile enzyme for many molecular biological applications. It catalyzes the hydrolysis of ester bonds in phosphomonoesters and is used in recombinant DNA technology to remove 5-phosphate groups from DNA fragments to prevent self-litigation of vector DNA molecules. Coupled to antibodies or oligonucleotides, it replaces radioactively labeled compounds by serving as a reporter and signal amplifying enzyme which cleaves chromogenic or fluorogenic substrates in diagnostic applications for the specific detection of DNA (Southern blot: E.M. Southern (1975) *J. Mol Biol.* 98, 503-517) or proteins (Western blots: W.N. Burnett (1981) *Anal. Biochem.* 112, 195-203).

Predominantly, AP is isolated from calf intestine (CIP) or the bacterium E coli. (BAP). AP consists of a homodimer. The stability of the enzyme, of advantage in diagnostic applications, can lead to severe problems in cloning experiments. Residual AP activity from the dephosphorylation of vector DNA can result in dephosphorylation of the DNA to be inserted so that no or only low yields of ligation products are obtained. Heat inactivation very often is not sufficient so that time-consuming removal is necessary using treatment with proteinase K and subsequent extraction from phenol/chloroform. This lengthy procedure will also drastically reduce the yield of the product. Alteratively, AP isolated from species living at low temperatures (shrimps) are employed; here heat inactivation is possible, however, reduced stability is disadvantageous for diagnostic applications.

A modified BAP derived from E. coli was genetically designed with a his_6 tail at its carboxy terminus. The his_6 tail was introduced using inverse PCR by which six histidine codons followed by a stop codon were placed at the 3' end of the gene (E. Blum et al. (1994) *Biochem Biophys J.* 29, 113-121). To achieve high expression levels of the recombinant enzyme in E. coli, the region coding for the signal peptide of AP together with the untranslated 5' and 3' regions were exchanged with homologous sequences from the E. coli ompA gene. The expression of the resulting protein construct was under the control of the IPTG (β-D-isopropyl-thio-galactoside) inducible ptac-promoter.

The BAP-his_6 synthesized in the E.coli cell can easily be isolated from an unpurified cell extract through affinity chromatography using commercially available Ni-NTA resins (Qiagen) to which it forms a strong and specific chelate complex via its his_6 tail. The modified enzyme is therefore now available in high yields, high purity and reproducible batch-to-batch quality. As part of the inventive process, BAP-his_6 is able to form with chelate-modified nucleic acids, a stable complex which for the first time makes available specific conjugates between proteins (here BAP) and nucleic acids in a reproducible 1:1 stoichiometry.

When peptides are generated by chemical synthesis, the his_6 tail can be directly incorporated during peptide synthesis. Chemical synthesis of peptides also allows the alternative approach in which a chelator functionality is attached to the synthetic peptide either at the N- or C- terminus or one of the side chains depending on which part of the peptide sequence is needed for the biochemical function.

The nucleic acid molecule can be functionalized either with the imidazoyl moieties or with the chelator functionalities. In case of synthetic oligonucleotides the chelator functionality can be introduced in different ways. An amino acid such as serine, cysteine or lysine can be transformed into a β-cyanoethylphosphoamidite (N.D. Sinha, J. Biernat, J. McManus and H. Köster (1984) *Nucleic Acids Res.* 12, 4539-4477) carrying a precursor of the chelator functionality (e.g. NTA as described in Figure 5 and 6 with serine as starting material). During deprotection after solid phase oligonucleotide

synthesis, the three carboxyl groups are liberated forming a NTA (nitrilotriacetic acid) group linked through a phosphodiester bond to the oligonucleotide chain. In yet another way, Figure 7 shows the introduction through a heterobifunctional trityl group. The oligonucleotide is, after regular final detritylation, retritylated with a heterobifunctional trityl group bearing an active ester moiety derived from either e.g. N-hydroxysuccinimide or employing active esters such as p-nitrophenyl esters. The active ester functionality is then reacted with a chelator molecule derived from e.g. lysine.

The imidazolyl functionality can be introduced during oligonucleotide synthesis employing an appropriate β-cyanoethylphosphoamidite as shown in Figure 8; single or multiple imidazolyl residues can be incorporated. A imidazolylnucleoside as shown in Figure 9 or a histidine peptide sequence covalently attached to the oligonucleotide chain (Figure 10) can also be used to introduce the necessary imidazolyl moieties for interaction with the chelator functionality.

The chelator and oligoimidazolyl functionalities can also be introduced in high molecular weight nucleic acids using either DNA dependent DNA or RNA polymerases or RNA dependent DNA polymerases using appropriately modified nucleoside triphosphates (either NTPs, 2'-dNTP, 3'-dNTPs, ddNTPs) as depicted in Figure 11. The base will carry either the chelator or the oligoimidazoyl functionality (Figure 12) in case of pyrimidine bases at C5 and in case of purine bases at C8 so that Watson-Crick base pairing is possible. Using the appropriate nucleoside triphosphates those functionalities can either be introduced internally (NTP for RNA synthesis or 2'-dNTP for DNa synthesis) or at the 3'-end (3'-dNTP for RNA synthesis, ddNTP for DNA synthesis). The incorporation can be performed during amplification procedures such as PCR, SDA or during DNA sequencing. Those skilled in the art will realize other approaches to introduce either chelator or oligo-imidazolyl moieties into nucleic acids.

Detection of the immobilized nucleic acid-protein/peptide conjugates can be achieved either directly on the polymer support or after selective cleavage of either reversible bond I (I') or II (II'). The signal can be detected by any of a number of means

including radioactivity, fluorescence, chemiluminescence (using e.g. 1,2-dioxetan derivatives) or colorimetric (using e.g. BCIP/NBT) methods depending on the substrates used as C or D Fig. 1, 2 and 3). D can be an enzyme such as AP which triggers upon contact with a substrate through its enzymatic activity the signal generation. C and D can also be detected through their molecular weight by employing mass spectrometric methods. Preferred mass spectrometer formats for use in analyzing the translation products include ionization (I) techniques, including but not limited to matrix assisted laser desorption (MALDI), continuous or pulsed electrospray (ESI) and related methods (e.g. Ionspray or Thermospray), or massive cluster impact (MCI); these ion sources can be matched with detection formats including linear or non-linear reflectron time-of-flight (TOF), single or multiple quadrupole, single or multiple magnetic sector, Fourier Transform ion cyclotron resonance (FTICR), ion trap, and combinations thereof (e.g., ion-trap/time-of-flight). For ionization, numerous matrix/wavelength combinations (MALDI) or solvent combinations (ESI) can be employed. Subattomole leels of protein have been detected, for example, using ESI (Valaskovic, G.A. et al., (1996) Science 273: 1199-1202) or MALDI (Li, L. et al., (1996) J. Am. Chem. Soc. 118:1662-1663) mass spectrometry.

The process of the invention is further demonstrated by solid phase separation and detection of Ligase Chain Reaction (LCR) products as seen in Figure 13 and products of PCR reactions (Figure 14). To those skilled in the art it is obvious that all applications and variations of amplification procedures including those useful for the detection of mutations and DNA/RNA sequencing are all adaptable to the process of the invention thereby significantly improving such processes.

The present invention is further illustrated by the following Examples, which are intended merely to further illustrate and should not be construed as limiting. The entire contents of all cited references (including literature references, issued patents, published patent applications and co-pending patent applications, as cited throughout this application) are hereby expressly incorporated by reference.

Example 1 BAP-his_6 Fusion Protein

The phoA gene coding for the BAP of E. coli (P.E. Berg (1981) *J. Bacteriol.* 146, 660-667; C.N. Chang et al. (1986) *Gene* 44, 121-125) was derived from E. coli strain HB101. The his_6 fusion at the carboxyterminus was generated via inverse PCR with six his codons followed by a stop codon derived from plasmid pHis 1 (E. Blum et al. (1994) *Biochem. Biophys. J.* 29, 113-121).

To increase the expression rate of the recombinant BAP-his_6 protein, its reading frame was embedded in the untranslated regions of the E. coli ompA gene (Chen et al. (1991) *J. Bacteriol.* 173, 4578-4586), coding for protein OmpA, which is a major protein constituent of the outer membrane in Gram-negative bacteria. In addition, the signal peptide of BAP (H. Inouye and J. Beckwith (1977) *Proc. Natl. Acad. Sci. USA* 74, 1440-1444) and the first two amino acids of the mature protein were replaced by the OmpA leader peptide and the first amino acid residue of mature OmpA, resulting in a mature chimeric BAP with the amino acid alanine instead of arginine-threonine at its N-terminus.

To bring the expression of the chimeric BAP-his_6 under the control of IPTG inducible chimeric tac-promoter (T. Amann et al. (1983) *Gene* 25, 167-178), a 2.5 kb *EcoRI-PstI* fragment containing the complete open reading frame of the ompA-phoA chimera) and the untranslated regions from the ompA gene was cloned into the expression vector pHK236 (a derivative of pJF118u: Fürste et al. (1986), kindly provided by M. Kröger, Giessen) to generate the BAP-his_6 expression plasmid vector pBAPHIS8. Expression is achieved by induction of logarithmic E. coli culture harboring plasmid pBAPHIS8 with IPTG in a final concentration of 1 mM for 2 h under shaking in a 37°C incubator. Isolation of BAP-his_6 is carried out according to developed protocols on Ni-NTA-Agarose (E. Hochuli et al. (1987) *J. Chromatography* 411, 177-184).

Example 2 Dephosphorylation of DNA Fragments with Solid Phase Bound BAP-his_6

A solution containing DNA fragments is incubated with beads carrying immobilized metal ions complexed with BAP-his_6 protein. To remove the enzymatic activity after the reaction is carried out, filtration or centrifugation removes beads with adsorbed enzyme. Alternatively, a solution containing DNA fragment can be filtered through a derivatized membrane, carrying immobilized metal ions complexed with BAP-his_6 protein.

Example 3 Detection of LCR Products in Microtiter Filter Plates

The use of BAP-his_6 as a reporter enzyme for LCR is carried out in the wells (96 or more) of a microtiter filter plate (MTFP) with 96 samples with oligos A-D (Figure 13). One of the oligos (oligo A being the marker oligo, Fig. 13) carries at its 5'-end a chelating group. In the presence of a template DNA the marker oligo is incorporated into one strand, the marker strand, consisting of oligos A and B, with B ligated to the 3'-end of oligo A. Under denaturing conditions (or after denaturing), ligation products, oligos and other smaller by-products are transferred by suction into a second MTFP with a derivatized filter membrane. To this filter, oligo D or part of it with sequence complementary to oligo B is coupled via NHS-DMT (heterobifunctional trityl derivative) linkage. Hybridization occurs between membrane bound oligo D and oligo B or the marker strand AB. After removal of supernatant and washing, only oligo A incorporated in the marker strand AB by ligation remains in the wells of the MTFP. BAP-his_6 and a divalent cation such as Ni^{2+} are incubated in the wells under adequate conditions to allow coupling of BAP-his_6 to the marker strand. After removal of unbound BAP-his_6 by washing and filtration, chromogenic or fluorescent AP substrates are added. Only wells containing the LCR product show AP activity as a positive result, bound D alone or the single strand CD cannot give rise to any signal. The experimental setup allows multiplex LCR by employing a mixture of oligos in the LCR and subsequent transfer of the LCR products by suction through a stack of different MTFP with specific bound oligo sequences. This experiment setup is amenable to automation, since the

reaction can be carried out e.g. in filter tubes or filter plates, which allow removal of contaminating agents, buffer changes and even detection *in situ* by dispensing and filtration of different liquids.

Example 4 Sequence Specific Detection of PCR Fragments

PCR is carried out in crude cell lysates with a derivatized oligonucleotide primer (Figure 14). After denaturing, the PCR reaction is filtrated through a membrane derivatized with a capture oligo. It can contain any sequence, which is complementary to the expected PCR fragment and hybridizes with strand elongated from derivatized oligo. Although any nucleic acid containing the sequence complementary to the capture oligo will be retained on the membrane, only PCR products containing the derivatized oligonucleotide primer can bind the modified BAP-his_6 enzyme. The PCR product is detected by BAP activity retained on the membrane after adequate washing procedure. This setup allows PCR with crude lysates, since contaminating agents can be removed by filtration and only the PCR products retained by hybridization to the membrane bound oligonucleotide give rise to a detectable signal. This setup is also amenable to multiplexing (see above).

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of the invention and are covered by the following claims.

We claim,

1. A composition comprised of at least two biopolymers conjugated to an insoluble support by at least one reversible linkage.

2. A composition according to claim 1, wherein the at least two biopolymers are comprised of nucleic acids.

3. A composition according to claim 1, wherein the at least two biopolymers are comprised of polypeptides.

4. A composition according to claim 1, wherein the at least two biopolymers are comprised of a nucleic acid and a protein.

5. A composition according to claim 1, wherein the at least one reversible linkage is formed through a trityl derivative, a chelate complex, a hydrophobic interaction or a photocleavable functionality.

6. A composition according to claim 1, wherein the insoluble support is selected from the group consisting of: a flat surface, a comb and a bead.

7. A composition according to claim 6, wherein the insoluble support is selected from the group consisting of: a silicon wafer, glass plate, metal, plastic, film and composites thereof with pits or wells.

8. A composition according to claim 7, wherein the biopolymer is conjugated to the insoluble support in an array format.

9. A composition according to claim 7, wherein the bead is comprised of an inorganic material selected from the group consisting of: silica, Controlled Pore Glass (CPG), plastic, metal, cellulose, Sepharose and Sephadex.

10. A composition according to claim 6, wherein the insoluble support is comprised of a magnetic or electromagnetic material.

11. A composition according to claim 2, wherein the nucleic acid is selected from the group consisting of: deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or analogs or mimetics of DNA or RNA.

12. A compostion according to claim 3, wherein the polypeptide is selected from the group consisting of an antibody, enzyme, receptor or peptide.

13. A composition according to claim 1, which contains a spacer between the biopolymer and the insoluble support.

14. A composition according to claim 4, which is made by the formation of a chelate complex between the nucleic acid and the polypeptide.

15. A composition according to claim 14, wherein the chelate complex is formed by the reaction of a nucleic acid containing a chelate functionality with a polypeptide containing an imidazoyl functionality in the presence of a metal ion.

16. A composition of claim 14, wherein the chelate complex is formed by the reaction of a nucleic acid containing an imidazoyl functionality with a polypeptide containing a chelate functionality in the presence of a metal ion.

17. A composition according to claim 15 or 16, wherein the polypeptide is an enzyme.

18. A composition according to claim 17, wherein the enzyme is an alkaline phosphatase.

19. A method according to claim 18, wherein the enzyme is bacterial

alkaline phosphatase (BAP).

20. A method for making a composition of claim 1, comprising the steps of:

a) immobilizing a nucleic acid to an insoluble support via a first reversible linkage; and

b) conjugating said nucleic acid with a polypeptide via a second reversible linkage.

21. A method according to claim 20, wherein the first or second reversible linkage is formed through a trityl derivative, a chelate complex, a hydrophobic interaction or a photocleavable functionality.

22. A method according to claim 20, wherein step b), the first or second reversible linkage forms a chelate complex.

23. A method according to claim 22, wherein the first or second reversible linkage is formed by the reaction of a nucleic acid containing a chelate functionality with a polypeptide containing an imidazoyl functionality in the presence of a metal ion.

24. A method according to claim 22, wherein the first or second reversible linkage is formed by the reaction of a nucleic acid containing an imidazoyl functionality with a polypeptide containing a chelate functionality in the presence of a metal ion.

25. A method according to claim 20, wherein the first or second reversible linkage are formed from functionalities or precursors, which are introduced into the nucleic acid during enzymatic synthesis.

26. A method according to claim 25, wherein the enzymatic synthesis

is part of an amplification procedure.

27. A method of claim 26, wherein the amplification procedure is selected from the group consisting of the polymerase chain reaction (PCR), the ligase chain reaction (LCR) and strand displacement amplification (SDA)..

28. A method according to claim 25, wherein the enzymatic synthesis is part of a nucleic acid sequencing procedure.

29. An oligonucleotide analog comprised of a β-cyanoethylphosphoamidite functionality with a chelate functionality.

30. An oligonucleotide analog of claim 29, wherein the chelate functionality is a precursor of nitrilotriacetic acid derived from either serine, cysteine or lysine.

31. An oligonucleotide analog comprised of a heterobifunctional trityl group with a chelate functionality.

32. An oligonucleotide analog of claim 31, wherein the chelate functionality is a precursor of nitrilotrisacetic acid derived from serine, cysteine or lysine.

33. An oligonucleotide analog comprised of a β-cyanoethylphosphoamidite functionality with an imidazolyl functionality.

34. An oligonucleotide analog comprised of a heterobifunctional trityl group with a oligohistidyl or oligoimidazolyl sequence.

35. An oligonucleotide analog according to claim 34, wherein the oligohistidyl sequence is present at the 5'- or 3'- terminus.

36. An oligonucleotide analog comprised of an imidazolylnucleoside-β-cyanoethylphosphoamidite.

37. A member selected from the group consisting of: nucleoside triphosphates, 2'-deoxynucleoside triphosphates, 3'-deoxynucleoside triphosphates and 2',3'-dideoxynucleoside triphosphates, wherein the member contains a chelate functionality at either C5 in the pyrimidine ring of thymine, uracil, or cytidine or at C8 in the purine ring of adenine, guanine or hypoxanthine.

38. A member selected from the group consisting of: nucleoside triphosphates, 2'-deoxynucleoside triphosphates, 3'-deoxynucleoside triphosphates and 2',3'-dideoxynucleoside triphosphates, wherein the member contains an oligohistidyl or oligoimidazolyl chain at either C5 in the pyrimidine ring of thymine, uracil, or cytidine or at C8 in the purine ring of adenine, guanine or hypoxanthine.

39. A recombinant protein which carries at its C-terminus an oligopeptide chain, which is capable of forming a chelate complex in the presence of metal ions.

40. A recombinant protein according to claim 39 which has enzymatic activity.

41. A recombinant according to claim 40, which is an alkaline phosphatase, which has an alanine residue at its N-terminus instead of arginine-threonine and which has at its C-terminus a chain of six histidine residues.

42. A peptide which carries at its N- or C- terminus an oligohistidyl sequence, which is capable of forming a chelate complex in the presence of metal ions.

43. A peptide which carries at its N- or C- terminus a chelator functionality which is capable of forming a chelate complex in the presence of metal ions.

44. A composition of claim 1, wherein the insoluble support is linked via a spacer to the nucleic acid through a reversible heterobifunctional trityl group and the nucleic acid is conjugated to an enzyme through a reversible chelate functionality.

45. A scomposition according to claim 44 in which the polymer support is comprised of magnetic beads, the chelate complex is formed via the nitrilotriacetic acid functionality in the presence of Ni^{2+} and the enzyme is BAP-his_6.

46. A composition according to claim 44 in which the polymer support is a silicon wafer carrying the reversible functionalities to bind the nucleic acid either directly on the surface or through beads in pits or wells in an array format, the chelate complex is formed via nitrilotrisacetic acid functionality in the presence of Ni^{2+} and the enzyme is BAP-his_6.

47. A composition according to claim 44 in which the polymer support is the filter bottom in the wells of a microtiter filter plate, the chelate complex is formed via nitrilotrisacetic acid functionality in the presence of Ni^{2+} and the enzyme if BAP-his_6.

48. A method of using the composition according to claim 44 to purify and to detect products of nucleic acid amplification procedures.

49. A method of claim 48, wherein the amplification procedure is selected from the group consisting of: the polymerase chain reaction, the ligase chain reaction and strand displacement amplification.

50 A method for using the composition according to claim 44 for determining the sequence of a nucleic acid.

51. A method for using the composition according to claim 44 to purify and to detect the identity and relative quantity of mRNAs or their corresponding

cDNAs for genetic or expression profiling.

52. A method for using the composition according to claim 44 to purify and to detect products of nucleic acid amplification procedures.

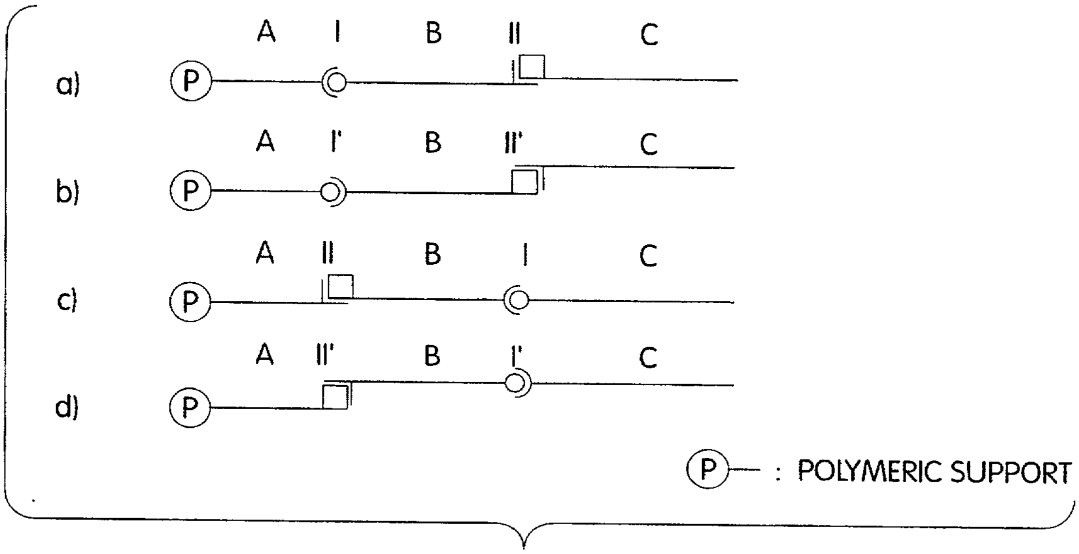


Fig. 1

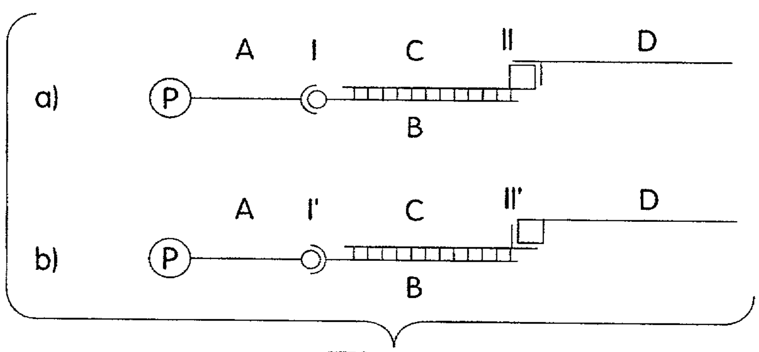


Fig. 2

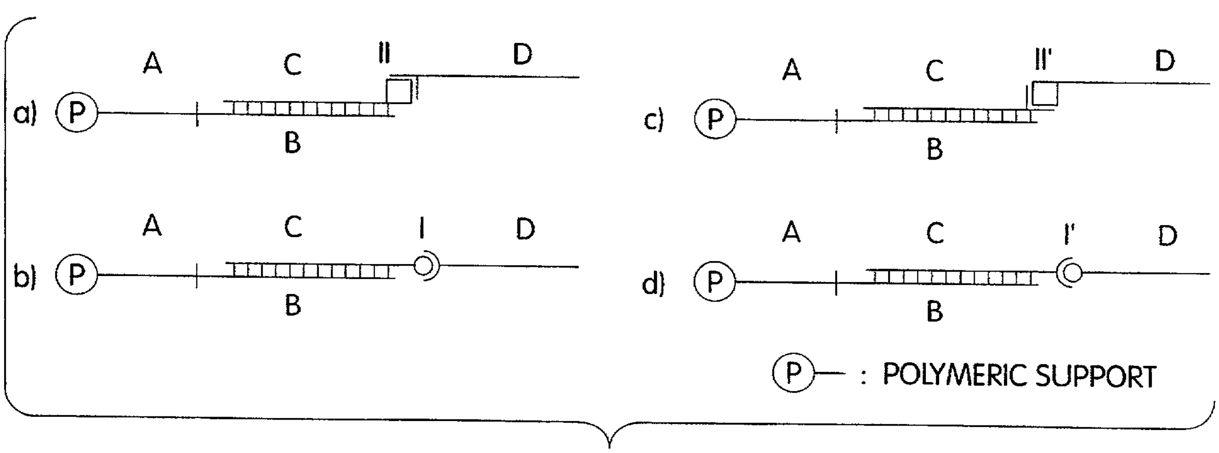


Fig. 3

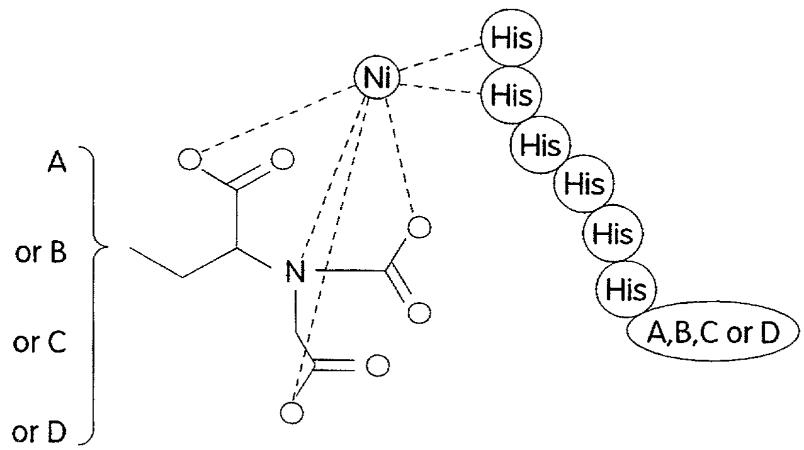
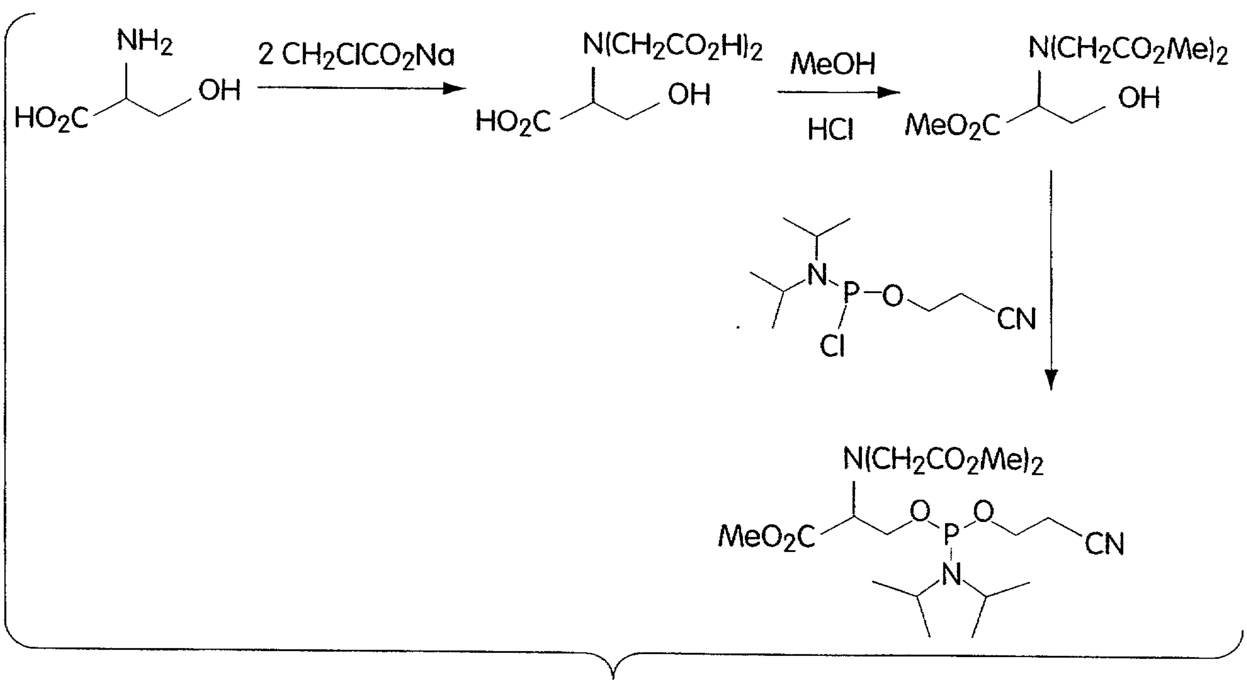

Ni
His
His
His
His
His
His
A,B,C or D
A
or B
or C
or D
N

Fig. 4


NH2
HO2C
OH
2 CH2ClCO2Na
N(CH2CO2H)2
HO2C
OH
MeOH
HCl
N(CH2CO2Me)2
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OH
N
P
O
Cl
CN
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MeO2C
O
P
O
CN
N

Fig. 5

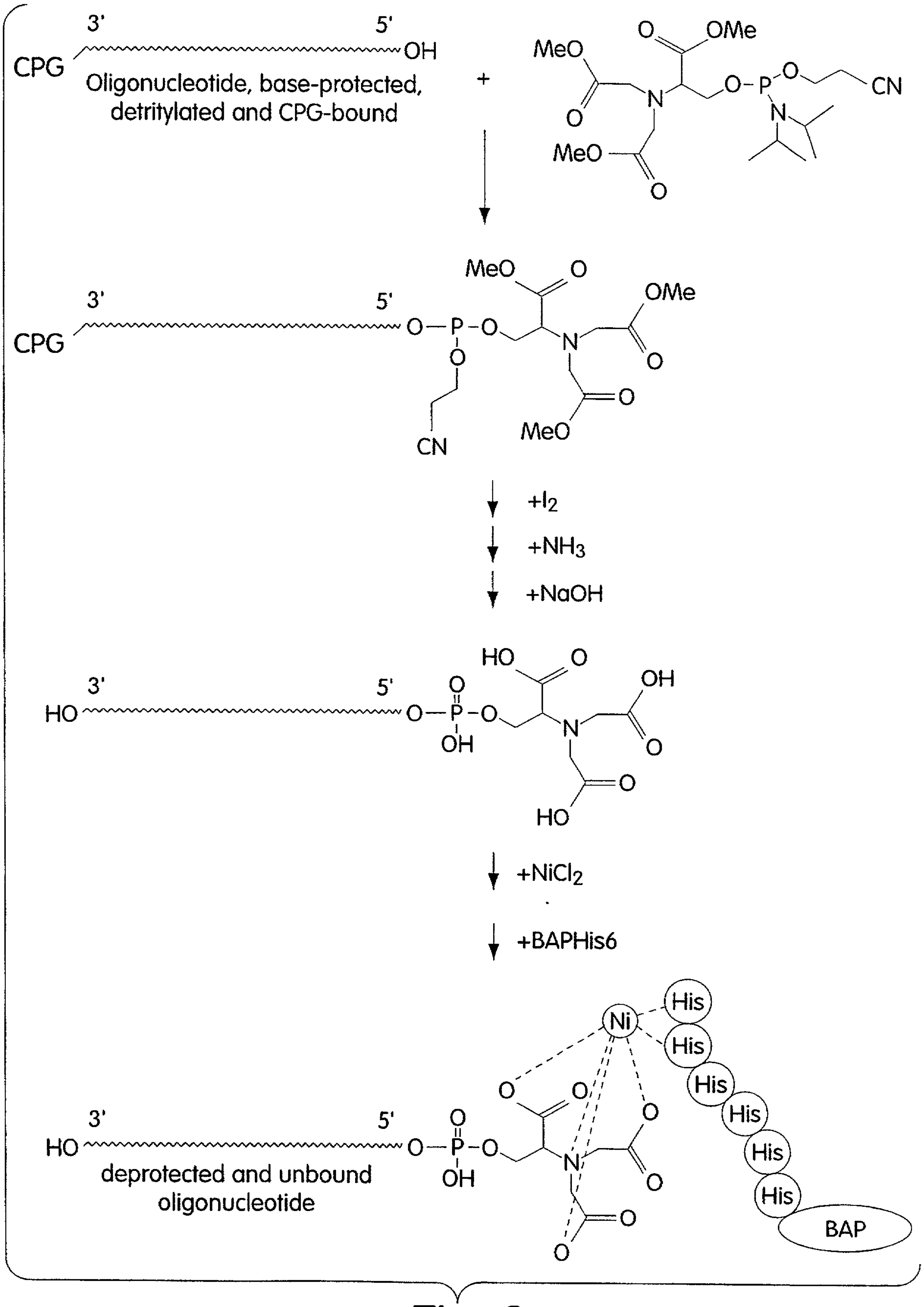

3'
5'
CPG
OH
Oligonucleotide, base-protected, detritylated and CPG-bound
+
MeO
O
OMe
O
N
O-P
O
CN
N
MeO
+I2
+NH3
+NaOH
HO
OH
+NiCl2
+BAPHis6
Ni
His
BAP
deprotected and unbound oligonucleotide

Fig. 6

09/355705

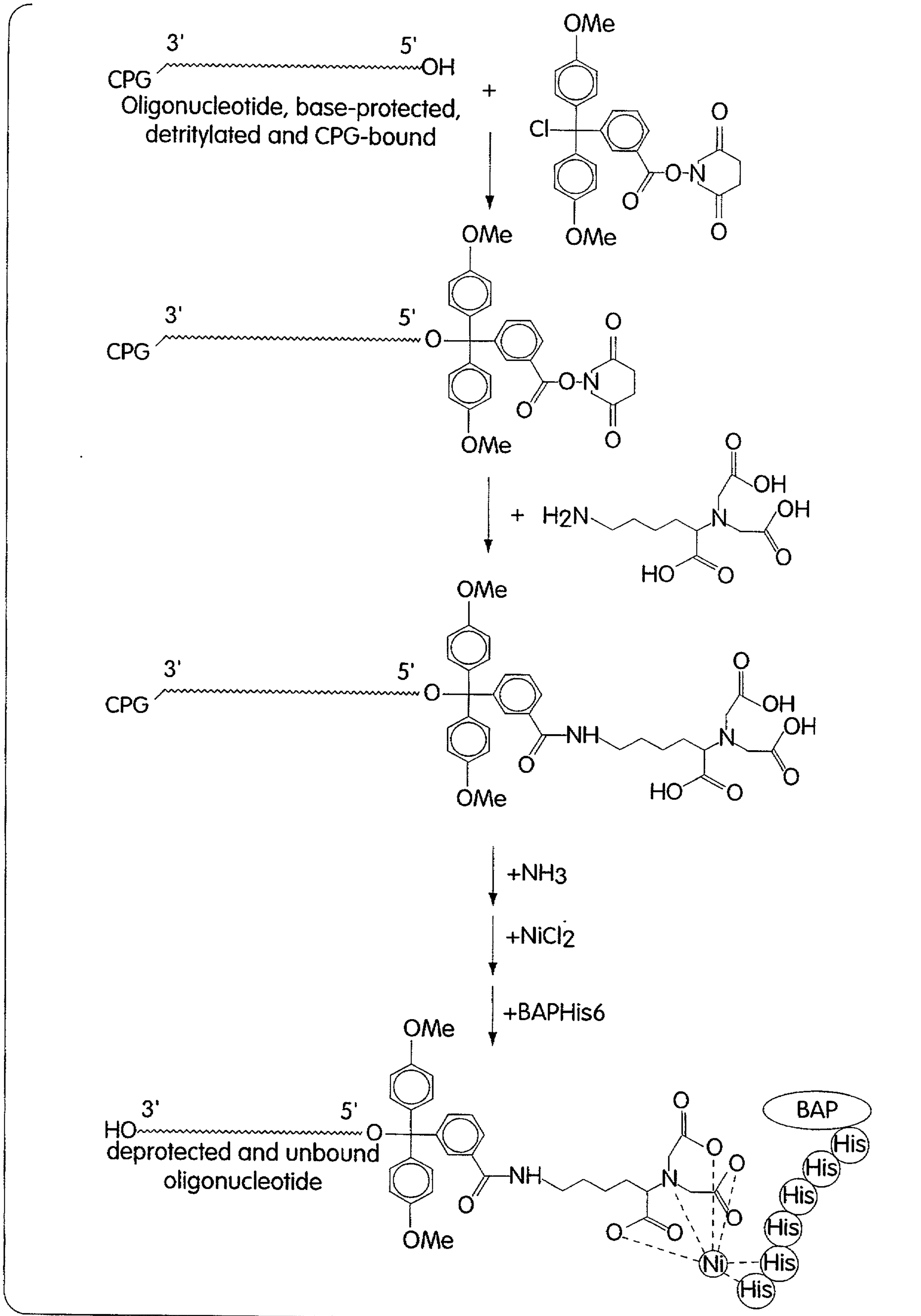
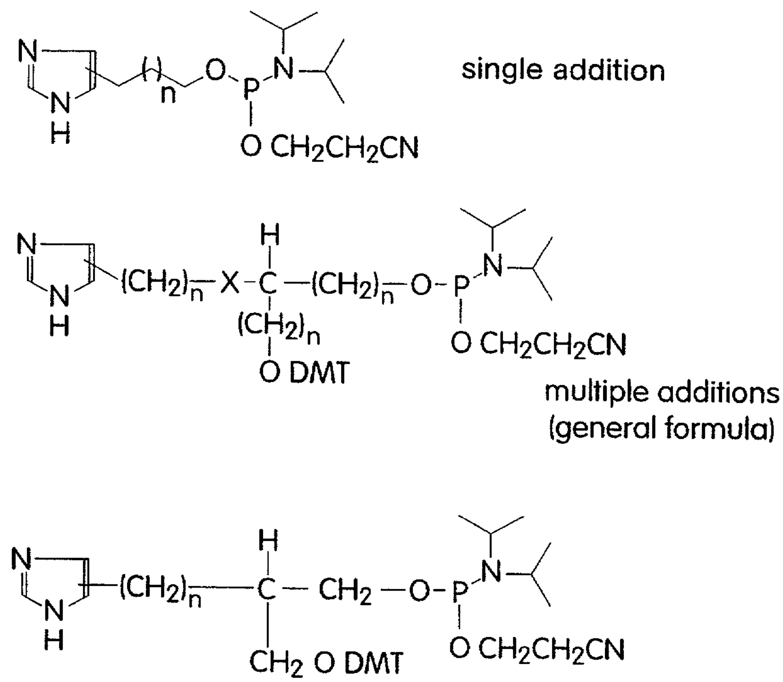


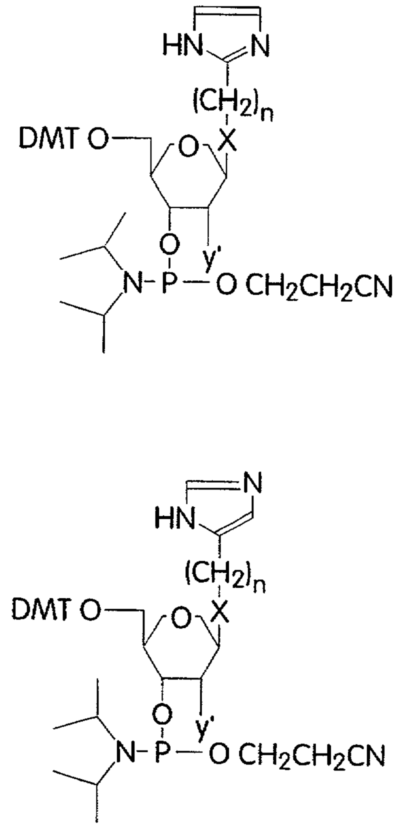
Fig. 7



X : - CH_2 - , - S - , - O - , - NH - , - NR - , - $\overset{O}{\overset{\|}{C}}$ - NH - , - $\overset{O}{\overset{\|}{C}}$ - O -

n : 0 - 20

Fig. 8



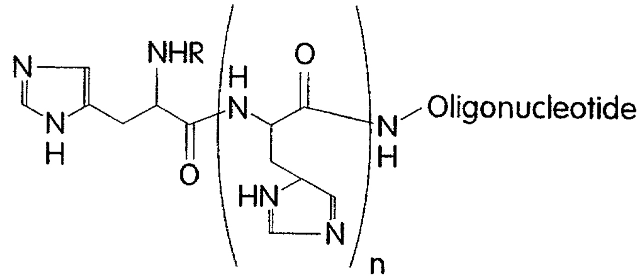
X : - S - , - O - , - NH - , - NR - , $-\overset{O}{\overset{\|}{C}}-$ NH - , $-\overset{O}{\overset{\|}{C}}-$ O -

n : 0 - 20

y' : - OR , - NHR , - SR , - F , - Cl , - Br , - O Me

R : Protecting Group (Lower alkyl, silyl alkyl, THP, etc.

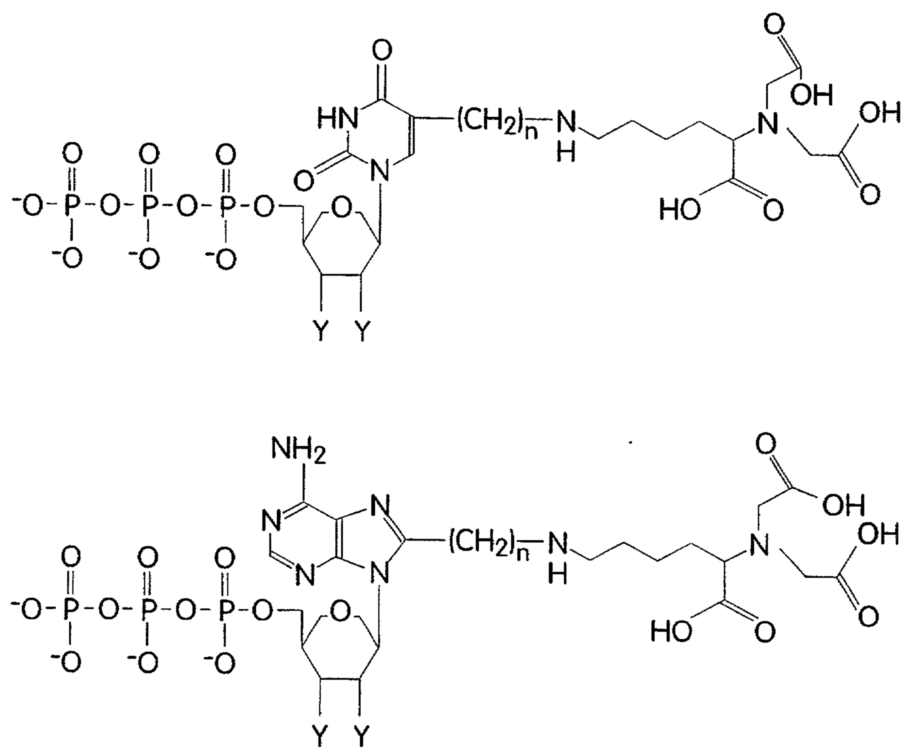
Fig. 9



R : Protecting Group (e.g. Fmoc , tBoc)

n : 0 - 20

Fig. 10



y : H , OH

n : 0 - 20

Fig. 11

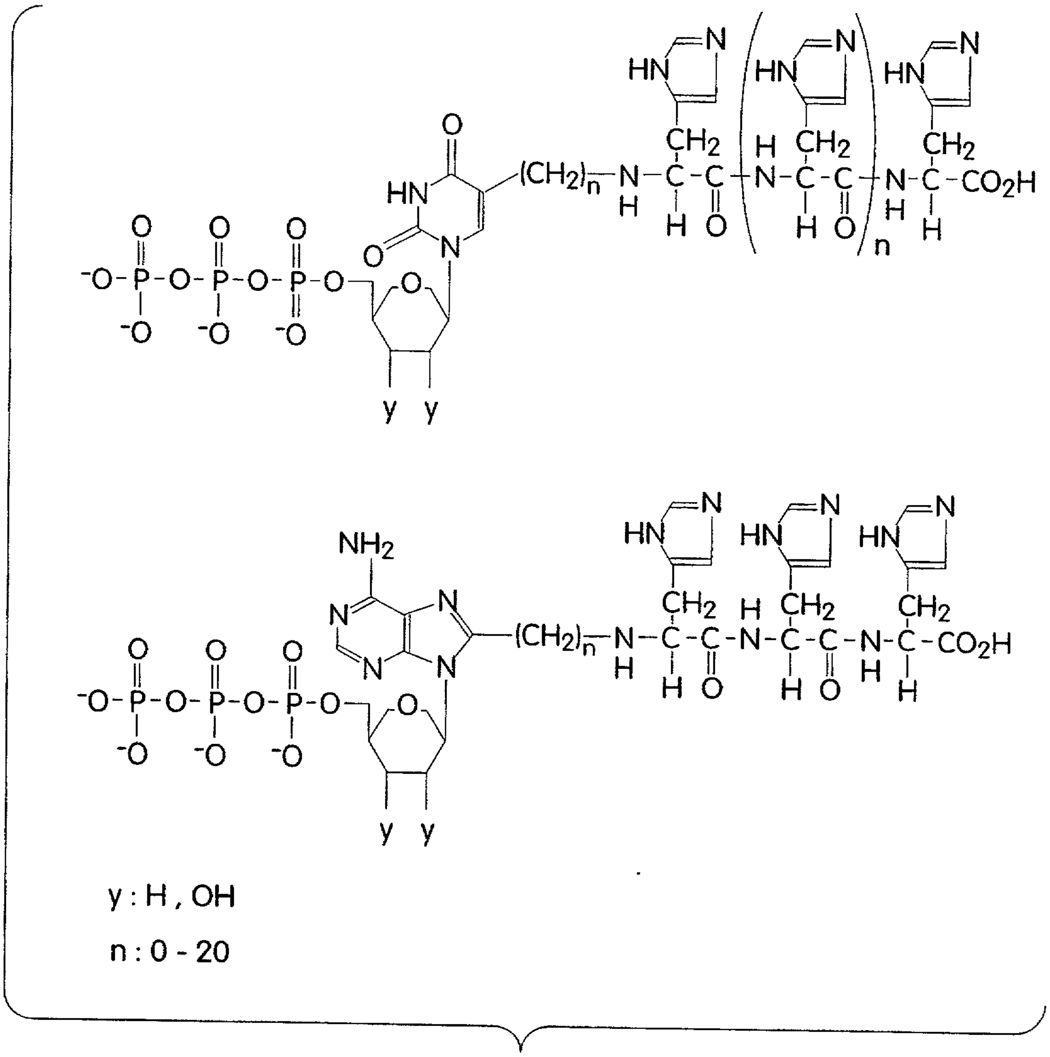


Fig. 12

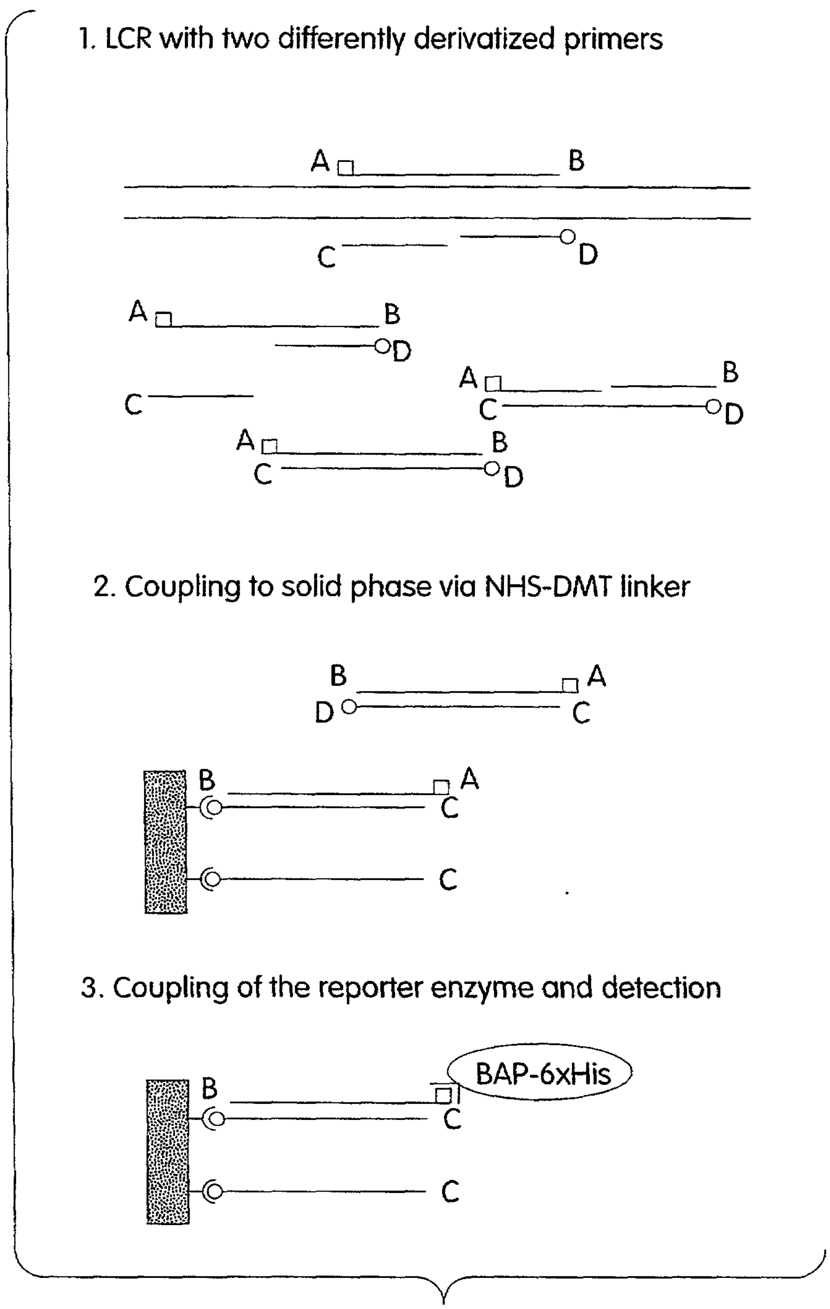

1. LCR with two differently derivatized primers
A
B
C
D
A
B
D
C
A
B
C
D
A
B
C
D
2. Coupling to solid phase via NHS-DMT linker
B
A
D
C
B
A
C
C
3. Coupling of the reporter enzyme and detection
BAP-6xHis
B
C
C

Fig. 13

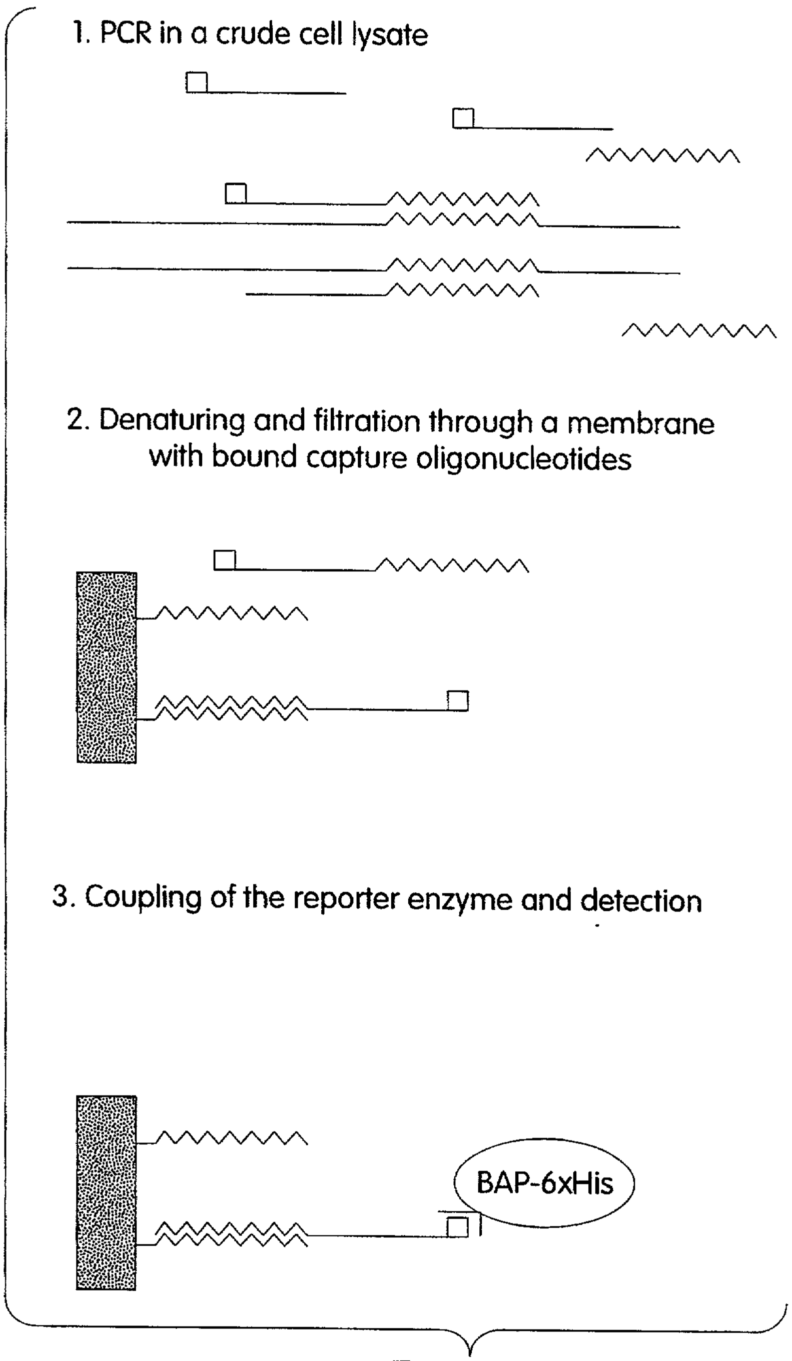

1. PCR in a crude cell lysate
2. Denaturing and filtration through a membrane with bound capture oligonucleotides
3. Coupling of the reporter enzyme and detection
BAP-6xHis

Fig. 14

DECLARATION FOR PATENT APPLICATION

As below-named inventors, we hereby declare that:

Our residences, post office addresses and citizenships are as stated below next to our names.

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A REVERSIBLE STOICHIOMETRIC PROCESS FOR CONJUGATING BIOMOLECULES

the specification of which

( ) is attached hereto.
( ) was filed by an authorized person on my behalf on _______ as Application Serial No. _________.
(X) was filed as PCT Application Serial No. PCT/US98/02007 on February 4, 1998, and was amended in a Preliminary Amendment filed herewith.

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

We hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below and so identified, or §365(a) of any PCT international application that designated at least one country other than the United States of America, listed below, and we have also identified below any foreign application for patent or inventor's certificate or PCT international application on this invention filed by us or our legal representatives or assigns and having a filing date before that of the application on which priority is claimed.

Number	Country	Day/Month/Year Filed	Priority Claimed (Yes or No)
N/A			

We hereby claim benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
60/037,165	February 4, 1997

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status
N/A		

PCT Application No.	Filing Date	Status
N/A		

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

We hereby appoint the following attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith and request that all correspondence and telephone calls in respect to this application be directed to Stephanie Seidman, HELLER EHRMAN WHITE AND McAULIFFE, 4250 Executive Square, Suite 700, La Jolla, California 92037:

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Attorney	Reg. No.
Stephanie Seidman	33,779
Paula K. Schoeneck	39,362
David A. Hall	32,233
Dale L. Rieger	43,045
Peng Chen	43,543
Gary H. Silverstein	39,372

Address for correspondence: Stephanie Seidman
HELLER EHRMAN WHITE AND McAULIFFE
4250 Executive Square, 7th Floor
La Jolla, California 92037

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Full name of joint inventor: Hubert Köster

Inventor's signature: [signature]

Date: 10/14/99

Residence: La Jolla, California CA

Post Office Address: 8636C Via Mallorca Drive,
La Jolla, California 92037, United States of America

Citizenship: Germany

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Full name of joint inventor: Andreas Ruppert

Inventor's signature:

Date:

Residence: Germany

Post Office Address: Hauptstrasse 10
35400 Linden GERMANY DEX

Citizenship: Germany

DECLARATION FOR PATENT APPLICATION

As below-named inventors, we hereby declare that:

Our residences, post office addresses and citizenships are as stated below next to our names.

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A REVERSIBLE STOICHIOMETRIC PROCESS FOR CONJUGATING BIOMOLECULES

the specification of which

( ) is attached hereto.

( ) was filed by an authorized person on my behalf on _______ as Application Serial No. ___ _______.

(X) was filed as PCT Application Serial No. PCT/US98/02007 on February 4, 1998, and was amended in a Preliminary Amendment filed herewith.

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

We hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below and so identified, or §365(a) of any PCT international application that designated at least one country other than the United States of America, listed below, and we have also identified below any foreign application for patent or inventor's certificate or PCT international application on this invention filed by us or our legal representatives or assigns and having a filing date before that of the application on which priority is claimed.

Number	Country	Day/Month/Year Filed	Priority Claimed (Yes or No)
N/A			

We hereby claim benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
60/037,165	February 4, 1997

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Application Serial No.	Filing Date	Status
N/A		

PCT Application No.	Filing Date	Status
N/A		

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full name of joint inventor: Hubert Köster

Inventor's signature:

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Full name of joint inventor: Andreas Ruppert

Inventor's signature: Andreas Ruppert

Date: Oct. 18, 1999

Residence: Germany
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